

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL
ANALYSIS OF THE CRUDE EXTRACTS OF THE AERIAL PART OF *AESCHYNOMENE*
UNIFLORA .MEY (FABACEAE)

BY

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NIGERIA

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Declaration

I declare that the work in this thesis entitled” Phytochemical Screening and Antimicrobial Analysis of the Crude Extracts of the Aerial Part of *Aeschynomene uniflora* has been carried out by me in the department of Chemistry. The information derive from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

Name of Student

Signature

Date

Certification

This thesis entitled “PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ANALYSIS OF THE CRUDE EXTRACT OF THE AERIAL PART OF *AESCHYNOMENE UNIFLORA*” by Jonathan Ilemona Achika meets the regulation governing the award of Master of Science Degree in Chemistry (organic) of the Ahmadu Bello University Zaria and is approved for its contribution to knowledge and literary presentation.

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Dedication

This work is dedicated to God Almighty

Acknowledgement

Thanks are to God almighty that provides all our needs, for giving me the wisdom, knowledge, sustenance and privilege to bring this work to completion.

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Abstract

The preliminary phytochemical screening of the aerial parts of *Aeschynomene uniflora* was carried out using standard method. The result of the phytochemical screening of crude petroleum ether, chloroform, ethyl acetate and methanol extracts revealed the presence of carbohydrate, cardiac glycoside, tannins, saponins flavonoids and anthraquinone. The antimicrobial screening against *Corynebacterium ulcerans*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Candida albicans*, *Candida stellatoidea* and *Candida krusei* was done using agar well diffusion method. The zone of inhibition of growth of microorganisms ranged from 16 - 18 mm for the petroleum ether extract, 22-27 mm for the chloroform extract, 22-26 mm for ethyl acetate extract and 19-22 mm for the methanol extract. The minimum inhibitory concentrations of the extracts were found to be between 15 mg/ml and 30 mg/ml while the minimum bactericidal /fungicidal concentration were found to be between 15 mg/ml and 60 mg/ml. The purification of the n-hexane fraction of the extract of *Aeschynomene uniflora* using a column of silica gel gave 3 β , 22E-Stigmasta-5, 22-dien-3-ol (C₂₉H₄₈O, 417 g/mol) (stigmasterol). The structure of the isolated compound was established based on spectral data (IR, 1D- and 2D- NMR) and comparison with literature. The compound is reported for the first time in this plant.

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ABBREVIATIONS

ID One dimensional

2D Two dimensional

TLC Thin Layer Chromatography

MIC Minimum Inhibition Concentration

MBC Minimum Bactericidal Concentration

MFC Minimum Fungicidal Concentration

IR Infrared

^{13}C NMR Carbon-13 Nuclear Magnetic Resonance

^1H -NMR Proton Nuclear Magnetic Resonance

DEPT Distortionless Enhancement Polarization Transfer

Cosy Correlation Spectroscopy

HMBC Heteronuclear Multiple Bond Coherence

HSQC Heteronuclear Single Quantum Correlation

NOESY Nuclear Overhauser Enhancement Spectroscopy

CHAPTER ONE

1.0 INTRODUCTION

There is a growing demand for herbal remedies for the world market despite the growth of the synthetic medicine production. At the same time, in the last decades there has been a sharp increase in the collection of some rare and endangered herbs from wild populations in many regions of the world (Manukyan, 2011). Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drugs because of the unmatched availability of the chemical diversity. Due to an increasing demand for chemical diversity, seeking therapeutic drugs from natural products, with interest particularly in edible plants has grown throughout the world. Botanicals and herbal preparations for medicinal usage contain various types of bioactive compounds (Sasidharan *et al.*, 2010). World Health Organisation (WHO) encourages the traditional drugs because of its fewer side effects and most of the European countries are expanding towards traditional medicines. Since ancient times, people have been exploring nature, particularly plants, in search of new drugs (Savithamma *et al.*, 2011). Plant materials have been used for the treatment of various diseases throughout the world before the advent of modern clinical drugs. The use of medicinal plants still plays an important role to cover the basic health needs in the developing countries and the industrialized societies had been traced to the extraction and development of several drugs from these plants as well as from traditionally used folk medicines (Shrikumar and Ravi, 2007). Various medicinal properties have been attributed to natural herbs; medicinal plants constitute the main source of new pharmaceuticals and health care products (Savithamma *et al.*, 2011).

Previously crude drugs were identified by comparison only with the standard descriptions available, but recently due to advancement in the field of pharmacognosy various techniques

have been used for the standardization of crude drugs (Savithramma *et al.*, 2010). Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, seeds, etc i.e., any part of the plant may contain active components (Cragg and David 2007). The World Health Organization (WHO) has described traditional medicine as one of the surest means to achieve total health care coverage of the world's population. In spite of the marginalization of traditional medicine practiced in the past, the attention currently given by governments to widespread health-care application has given a new impetus to research, investment and design of programmes in this field in several developing countries in Africa and elsewhere. Traditionally, rural African communities have relied upon the spiritual and practical skills of the TMPs (traditional medicinal practitioners) whose botanical knowledge of plant species and their ecology and scarcity are invaluable. Throughout Africa, the gathering of medicinal plants was traditionally restricted to TMPs or to their trainees. Knowledge of many species was limited to this group through spiritual calling, ritual, religious controls and, in southern Africa, the use of alternative names not known to outsiders (Cunningham, 1993). Medicinal plants contain some organic compounds which produce definite physiological actions on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edeoga *et al.*, 2005). Medicinal plants are of great importance to the health of individuals and communities (Edeoga *et al.*, 2005). Many of these indigenous medicinal plants are used as spices and food plants. They are sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes (Okwu, 2001).

1.1 Biological Background of Medicinal Plants.

All plants produce chemical compounds as part of their normal metabolic activities. These are arbitrarily divided into primary metabolites such as sugars and fats found in all plants, and secondary metabolites which are compounds that are not essential for basic functions. The secondary metabolites are found in a smaller range of plants or some useful ones found only in a particular genus or species (Patrick, 2005). The functions of secondary metabolites are varied. For example, some secondary metabolites are toxins to deter predators and others are pheromones used to attract insects for pollination. Phytoalexins protect plant against bacterial and fungal invasions, while allelochemicals inhibit rival plants that are competing for soil and light (Patrick, 2005). Plants regulate their biochemical paths in response to the local mix of herbivores, pollinators and microorganisms. The chemical profile of a single plant may vary over time as it reacts to changing conditions. It is the secondary metabolites and pigments that can have therapeutic actions in human which can be refined to produce drugs (Patrick, 2005). Alkaloids and other nitrogen containing cyclic compounds are plant poisons and drugs. Phenolics are benzene hydroxyl groups for example, anthocyanins, isoflavones and tannins. Terpenoids are built up from five –carbon isoprene units and so can also be called isoprenoids if it contains oxygen and is present in many spices, which are fragrances (Cunningham, 1993). The word “drug” comes from the Dutch word “droog” (from the French word *Drogue*) which means “dried plant”. Some examples are insulin from the roots of *dahlia*s, quinine from the *cinchona*, morphine and codeine from the poppy. Medicinal plants are plants whose extracts or parts can be used directly or indirectly for the treatment of different ailments. Therefore, the use of traditional medicine and medicinal plants in most developing countries as a basis for the maintenance of good health has been widely observed (Edward, 2001). Scientists throughout the

world are trying to explore the precious assets of medicinal plants to help the suffering humanity. Furthermore, today more than 30% of the pharmaceutical preparations are based on plants (Shinwari and Khan, 1998). However, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants. The use of medicines from plants in the form of local medicine dates back to 4000-5000 B.C, while the medicinal values of these plants are due to the presence of some active compounds which produce physiological actions in the human and animal body (Shrikumar and Ravi, 2007). Some of the important bioactive compounds found in medicinal plants are alkaloids, glycosides, resins, gums, mucilages etc. (Sack and Forehlich, 1982). It was observed that developed countries mostly import raw materials of valuable medicinal plants from developing countries. They are then screened, analyzed and used in drug preparations, and returned as high priced medicines to developing countries (Shinawie, 2002). In Pakistan there are about 2000 estimated species of medicinal plants out of which 400 are extensively used in traditional medicine. Pakistan has variety in climate and therefore rich in medicinal plants, but no systematic attempt has been made to work and utilize natural resources of this country. Interest was revived recently in the investigation of medicinal plants to identify novel active phytochemicals that might lead to drug development. Nature has generated such substances for millennia — before modern synthetic chemistry developed in the mid-19th century. Because these substances arise from a more or less hostile environment, the percentage of biologically active natural substances is relatively high in comparison with substances from artificial sources. Currently, more than 50% of drugs in clinical use have a natural-product origin, and about half of the world's 25 best-selling pharmaceutical agents are natural-product derived (Jin *et al.*, 2011).

1.2 History of Herbal Medicine

Herbal medicine sometimes referred to as botanical medicine or herbalism, involves the use of plants or parts of plants to treat injuries or illnesses. This field also covers the use of herbs or botanicals to improve overall health and wellness. Herbalist, herbal medicine practitioners, traditional medicine practitioners, ayurvedic, homeopathic, and naturopathic healers all use herbal remedies in their practices. Seeds, leaves, stems, bark, roots, flowers, and extracts of all of these have been used in herbal medicine over the millennia. These supplemental treatments have been delivered raw, in teas and tinctures, as topical applications, in liquid forms, and in pills and capsules. In the beginning the plants were consumed raw or combined with hot water as a soup or tea. Later, the plants were dried and crushed for other uses. The plants were found in the wild and uses were often based on superstitious or visual clues. Plants were often used to treat body systems because they were shaped like that body part or because they grew in a particular area. As science began to take a closer look at herbal remedies, their use became more refined. Herbs, and other plants, are actually the precursors to many of today's medicinal drugs. Some of the pharmaceutical medications on the market are extracts of some of these traditional herbs (The National Center for the Preservation of Medicinal Herbs 1998). Today, many modern and Western medicine practitioners are beginning to look at herbal remedies for some common and not-so common, disorders. The lower cost, and often safer use, has attracted many medical professionals. Some physicians use herbs to off-set the side effects of pharmaceuticals.

1.2.1 Herbal medicine today

Herbal medicines are still in use today. In some respects they have gained a new momentum in the medical field. As many people seek alternative treatments and begin to check out traditional and Eastern medicine, herbs are becoming more popular. As physicians seek new treatments for many common illnesses they are beginning to revisit the traditional remedies using herbal medicines. Pharmaceutical medications with their potentials for harmful side effects and addiction are becoming less popular. People are seeking alternatives to the modern medical interventions. Improving and maintaining health naturally is a very popular approach to overall wellness. The herbs used today are generally cultivated for those purposes. Very few herbs are harvested from the wild, with the exception of a few still found in the rainforests and higher elevations. The cultivation of herbs for medicinal uses is a large field and more people are beginning to plant their own herb gardens. Many monasteries continue to grow large herbal gardens within their walls. Elderly people also metabolize medications differently, and generally are on more medications, and therefore must also exercise caution when trying new herbal treatments. Underlying ailments that may affect the body's ability to process or absorb medications are also an issue (The National Center for the Preservation of Medicinal Herbs 1998). Herbal medicine has enjoyed a long, and colorful, history. From the early Chinese Empires to modern physicians' offices, herbal medicines have continued to be a part of the medical field. Herbal treatments have matured throughout history, along with the methods of delivering them. In the beginning, the herbs were used in a hit or miss method and required major events to change their use. Research and clinical trials have helped to shape the field of medicine, and the future for herbal medicine looks bright (The National Center for the Preservation of Medicinal Herbs 1998). The widespread use of herbal remedies and healthcare

preparations has been traced to the occurrence of natural products with medicinal properties (Gyawali, 2010). Increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (UNESCO, 1998). The percentage composition of the volatile components and characteristics of Volatile Organic Compounds (VOCs) provide an important parameter for the characterization of the plant (Paula *et al.*, 2001). Careful identification of VOCs for fragrance and pharmacologically active ingredients will show the presence of numerous useful compounds. They are gaining increasing interest because of their relatively safe status and their exploitation for potential multi-purpose functional use. Most of the constituents are terpenoids, generally monoterpenes and sesquiterpenes, as well as sometime diterpenes and aromatic compounds derivatives. The VOCs present in essential oils has been reported for their anti-spasmodic, restraining, diuretic, anti-biotal, antimicrobial, antifungal, insecticidal, and anthelmintic efficiency (Singh, 2004). Due to species climatic and geographical conditions, temperate and alpine plants of the Himalaya offer greater possibilities of having novel molecules and even largest quantities of the active compounds (Rajbhandari *et al.*, 2001)

1.3 Justification of the Research

The choice of *Aeschynomene uniflora* as the plant of interest in this work is based on its claimed ethno medicinal uses among traditional medicine practitioners in the tropics, including West Africa. Therefore, there is the need for a scientific study to justify or otherwise the medicinal potentials of this plant and identify the active ingredients

1.4 Scope and Limitation of This Study

The research thesis is limited to phytochemical screening, antimicrobial screening, isolation, characterization and structural elucidation of the active principle of *Aeschynomene uniflora*

1.5 Aim and Objectives

1.5.1 Aim

To determine the phytochemicals properties and the antimicrobial effects of crude extracts of *Aeschynomene uniflora* .Mey crude extracts on some pathogens.

1.5.2 Objectives

The objectives of this study are:

- (i) Extraction of the ground plant material using different solvents, from non-polar to polar ones
- (ii) Phytochemical screening of the crude extracts
- (iii) Antimicrobial screening of the extracts against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium ulcerans*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Candida albicans*, *Candida stellatoidea* and *Candida krrusei*
- (iv) Chromatographic purification of the extracts
- (v) Structural elucidation and characterization of the isolated compounds using available spectral techniques
- (vi) Testing the potency of the isolated components against the selected microorganisms.

CHAPTER TWO

Aeschynomene uniflora is an [erect](#) and [ascending plant](#), which is short-lived (2-4 years) and it is a [perennial shrub](#). It is 0.5 - 2 m tall and 1 - 2.5 m across, depending on ecotype. The stems are 1.5 - 2 mm in diameter in upper parts; to 4 mm, becoming hollow and pithy and more rigid with age. It is [glabrous](#) to [hispid](#) (bristles 1 - 2 mm long, glandular); its mature stems to 20 mm diameter, tending to woody. The Leaves are [pinnate](#), 3 - 8 cm long, it is grey green to dark green in color, often with purplish tinge; leaflets (pinnae) 8 - 38 pairs, 3 - 15 mm long, 1 - 3 mm wide. It is [linear](#) or [oblong linear](#); exhibiting nyctinastic (night) and thigmotactic (touch responsive) movement causing pinnae to fold on the rachis; rachis and [leaflet](#) margins and midribs often [ciliate](#). [Inflorescence](#) a loose, few-flowered [raceme](#), often branched; [corolla](#) varying in color from pinkish to pale mauve or yellow-orange, [standard petal](#) 5 - 10 mm long and wide, usually with red or purple striations. The [Pod](#) are mostly slightly curved, 3 - 9 segments each 2.5 - 5 mm wide and 3 - 6 mm long ([terminal](#) and basal segments longer due to retained [style](#) and [stipe](#)). The upper [suture](#) entire, the lower scalloped between joints; breaking into segments at maturity.

The seeds are kidney-shaped, 2 - 3 mm long and 1.5 - 2 mm wide, grey-green to light and dark brown or black. 150,000 - 300,000 seed-in-[pod](#)/kg or 350,000 - 500,000 dehulled seeds/kg. The [Hispid](#) stems and leaves emit distinctive odour due to glandular secretions (Bishop, 1992).

2.2 Traditional uses of *Aeschynomene uniflora*

The plant is used in traditional medicine in treatment of Psychotic disorder, tuberculosis, skin infections, antidote to snake venom, menstrual disorders and small pox. The aqueous extract of the whole plant is administered topically over the whole body to cure small pox in Northern Nigeria. The plant is eaten as vegetable to cure fever symptoms and cough in Benue State Nigeria.

2.3 The *Aeschynomene* Species

This species belongs to the family leguminosae which is a marshy erect herb. In traditional medicinal system, this plant is used to treat body pain and swellings (Yoganarasimhan, 2000; Wilson *et al.*, 2007). It is also used to treat mumps (Rajendar *et al.*, 2010). It is mainly used to treat joint pains (Padal *et al.*, 2010). The plant genus *Sesbania*, family Leguminosae, is comprised of about 60 species which are widely distributed throughout tropical and subtropical regions. Most species are annual herbs or shrubs, but a few are small trees (Burgess *et al.*, 1990). Many species of *Sesbania* are used for soil improvement as green manures or agro forestry trees (Palm *et al.*, 2001). *Sesbania grandiflora*, syn. *Aeschynomene grandiflora*, is a small erect tree that grows rapidly, is a sparsely branched tree that provides light shade, and is often grown as an ornamental (Staples *et al.*, 2005).

2.3.1 *Aeschynomene virginica*

Aeschynomene virginica is a rare species of flowering plant in the [legume family](#) known by the common names Virginia jointvetch and sensitive jointvetch. It is native to a small section of the East Coast of the United States, where it has a fluctuating annual global population scattered in

about 20 mostly small occurrences (Smith, 1986). Counts and estimates revealed two populations in [New Jersey](#) including several thousand individuals, one population of a few hundred plants in [Maryland](#), several variable and unstable populations in ditches in [North Carolina](#), and several populations including about 5000 individuals in [Virginia](#) (Carulli and Fairbrothers., 1988). Habitat alteration has reduced the amount of sites where the plant can grow. The plant became a federally listed threatened species of the United States in 1992 (Smith, 1986). Some confusion has occurred in the literature, specifically that this species is named as a [noxious weed](#) of [soybean](#) and [rice fields](#). The weedy species in question is actually [Aeschynomene indica](#), not the rare and threatened *A. virginica*. (Carulli and Fairbrothers., 2000). *A. virginica* is an annual herb which grows erect and can reach two meters in height. (Smith, 1986). The alternately arranged leaves are each made up of many pairs of hairy, glandular leaflets. The leaves are touch-sensitive, folding when touched. Blooming takes place in summer and early fall. The pea-like flower is about a centimeter long and yellow in color with stark red veining. The flowers are [pollinated](#) by insects such as the [Least Skipper](#) (*Ancylozypha numitor*) and [leaf-cutter bees](#) (family Megachilidae). The fruit is a [legume](#) pod up to 7 centimeters long which is narrowed between the seeds and easily segments. The segments float and the seeds may be [dispersed](#) on the water if they don't fall out immediately. Bits of the pods may get caught up in floating vegetation debris and can be deposited with it in new territory. (Carulli and Fairbrothers., 2000). This plant occurs in [freshwater tidal marshes](#) that experience [tidal action](#) but low [salinity](#). It grows in bare, disturbed areas that are mostly cleared of other vegetation, such as newly deposited shorelines or recently disturbed terrain dug out by [muskrats](#). It [germinates](#) in wet soils but not in submerged areas, and it is not successful in areas with thick vegetation. (Griffith and Forseth 2003). Interestingly, *Aeschynomene virginica* has frequently been confused in the

scientific literature with the invasive weed, *Aeschynomene indica*, and referred to erroneously as an agricultural pest! Recent genetic and taxonomic studies have resolved this confusion (Carulli and Fairbrothers, 1988).

2.3.2 *Aeschynomene aspera*

Aeschynomene aspera (Fabaceae) is a tall erect sub shrub in swampy areas, with stout glabrous nodular stems, full of white pith. It is commonly known as Sola pith plant (English) Sola (Hindi) Aatrunetti (Tamil). It is used as a substitute for cork material and as a sun hats fide. Pith is used as helmets and floats (Burnel and Henry., 1996). This species in North Bihar region is used as ornamental purpose and used for making berths (fish boats) also for several handicraft items like garlands, maur (head crown). In Ayurveda this species is known as Pashenabhed recommended for painful micturition and for breaking uric acid calculi. It is a weed of rice paddies (Caton, *et al.*, 2004). The roots are boiled in less quantity of water and made in to paste applied on mumps (Caton, *et al.*, 2004). Aerial part juice is given to cure cold, cough, and fever. Dried young shoot powder with half tea spoon powdered candy is given to increase the consistency of semen; local herbalists used it for urinary troubles (Panda and Mishra., 2011). *A.aspera* is also recognized as leafy vegetable (Varaprasad., 2011).

The hepatoprotective activity of benzene and alcoholic extracts of root of *Aeschynomene aspera* was investigated in rats for carbon tetrachloride induced hepatotoxicity and reported by (Thirupathy *et al.*, 2011).

2.3.3 *Aeschynomene indica*

This plant is a shrublets or annual herbs about 150 cm tall, its stems are erect and could be branched, cylindrical, hollow, glabrous, corky at base, often with nodule-bearing adventitious roots. Stipules elliptic to lanceolate, 4-11 × 1-2 cm, membranous, caducous, base auriculate, apex acuminate. Leaves 20-60-foliolate, often sensitive; petiole 2-4 mm; rachis with tuberculate-based trichomes; leaflet blades linear-oblong, 3-13 × 1-3 mm, papery, base oblique, apex obtuse and mucronate. Inflorescences axillary, racemose, sometimes short or reduced to a solitary flower; peduncle 4-7 mm, with tuberculate-based trichomes; bracts ovate, caducous, margin often denticulate. Bracteoles ovate-lanceolate, persistent. Calyx 3-4 mm, membranous, glabrous. Corolla pale yellow with purplish longitudinal striations. Legume linear-oblong, 2.2-3.4 cm × 3-5 mm, straight, herbaceous to leathery, abaxial suture straight, slightly indented; articles 2-8, quadrate, slightly mucronate and with tuberculate-based trichomes. Seeds blackish brown and this species is used for green fertilizer, medicinal purposes, and as an industrial raw material.

Aeschynomene indica is a swampy medicinal plant used to treat kidney stones and urinary disorders by the Chenchu tribes and the local herbalists. All plant parts Leaf, Flower and Fruits were screened out for their secondary metabolites in the four selected solvents and observed the presence of alkaloids, flavonoids, phenols, steroids and glycosides. Qualitative analysis of flavonoids (6 identified and 1 unidentified) phenols (19 identified and 5 unidentified) anthocyanidins (3) was carried out. Isochlorogenic acid, coumarin, p-hydroxy benzoic acid as the major phenolic compounds; kaempferol and apigenin as major flavonoids; delphinidin, malvidin and peonidin as the major anthocyanidins (Aruna *et al.*, 2012). Antimicrobial activity of the leaf cold water, hot water, methanol and alcohol extracts are more efficient on the selected four

pathogens of gram positive (*Bacillus subtilis*, *Staphylococcus aureus*) and gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria than the control drugs Ampicillin and Gentamycin with an average inhibitory zone of 20 mm to 41 mm at 10 mg/well. concentration of the drug. Minimum Inhibitory Concentrations were observed between 0.55- 0.75 mg/ml equal to that of the control drugs. (Aruna *et al.*, 2012).

Physicochemical parameters of *Aeschynomene indica* leaf powder were determined and reported as total ash, watersoluble ash, acid-insoluble ash, alcoholsoluble extractive, water-soluble extractive and moisture content (Anonymous, 1985).

2.3.4 *Sesbania grandiflora*

Sesbania grandiflora (also known as agati, syn. *Aeschynomene grandiflora*, *Agati grandiflora* or hummingbird tree/scarlet wisteria is a small tree in the genus [Sesbania](#). It is a fast-growing tree, leaves are regular and rounded and the flowers white and red in color according to its species. The fruits look like flat, long and thin green beans. The tree thrives under full exposure to sunshine and is extremely frost sensitive. (Joshi., 2008). Its a small soft wooded tree up-to 3-8m, leaves 15-30cm long; leaflets 10-20 pairs or more and an odd one. Oblong, 1.5-3.5cm long variety red, 7.5-10cm long in lax, 2-4 flower racemes, calyx campanulate, shallowly 2-lipped. Pods slender, falcate or straight, 30-45cm long, suture thick, Seeds ca. 30, to 8mm. Indigenous from Malaysia to North Australia; Cultivated in many parts of India. It has a large number of traditional uses.(Kirtikar and Basu., 2005). It contain arginine, cysteine, histidine, isoleucine, phenylalanine, tryptophan, valine, threonine, alanine, asparagine, aspartic acid, oleanolic acid, galactose, Rhamnose & glucuronic acid. *Leaves* used as tonic, diuretic, laxative, antipyretic, chewed to disinfect mouth and throat. *Flower* in headache, dimness of vision (Joshi., 2008)

Catarrh, Headache, cooling and improving appetite, bitter, astringent, acrid, antipyretic. *Bark* is used cooling, bitter tonic, anthelmintic, febrifuge, diarrhea, Small pox, Astringent (Prajapati *et al.*, 2003) *Fruits* in Bitter & acrid, laxative, fever, pain, bronchitis, anemia, tumors, colic, jaundice, poisoning. The root is used in rheumatism, expectorant, painful swelling, catarrh (Kirtikar *et al.*, 2005).

2.4 Phytoconstituents and Medicinal Uses of the *Aeschynomene* Species

2.4.1 General medicinal properties of the *aeschynomene* species

A.aspera aerial part juice is administered to cure cold, cough, and fever. The Dried young shoot powder with half tea spoon powdered candy is given to increase the consistency of semen; local herbalists used it for urinary troubles (Panda and Mishra, 2011). *A.aspera* is also recognized as leafy vegetable (Varaprasad, 2011). *Aeschynomene indica* is a swampy medicinal plant used to treat kidney stones and urinary disorders by local herbalists (Aruna *et al.*, 2012).

The leaves of *A. graniflora* are used as tonic, diuretic, laxative, antipyretic, chewed to disinfect mouth and throat. The flower in headache, dimness of vision catarrh, headache, cooling and improving appetite, bitter, astringent, acrid, antipyretic (Joshi, 2008). Bark is used as a bitter tonic, anthelmintic, astringent febrifuge, and for curing diarrhea, small pox, (Prajapati *et al.*, 2003). Fruits in Bitter & acrid, laxative, fever, pain, bronchitis, anemia, tumors, colic, jaundice, poisoning. Root used in rheumatism, expectorant, painful swelling, catarrh (Kirtikar *et al.*, 2005). The hepatoprotective activity of benzene and alcoholic extracts of root of *Aeschynomene aspera* was investigated in rats for carbon tetrachloride induced hepatotoxicity and reported by (Thirupathy *et al.*, 2011).

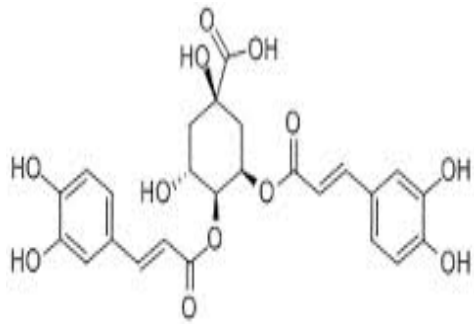
2.4.2 Phytochemical properties of the *Aeschynomene* species

All parts of the plants leaf, flower and fruits of *Aeschynomene indica* were screened out for their secondary metabolites in the four selected solvents and observed the presence of alkaloids, flavonoids, phenols, steroids and glycosides. Isochlorogenic acid (1), coumarin (2), para hydroxyl benzoic (3) acid as the major phenolic compounds; kaempferol (4) and apigenin (5) as major flavonoids; delphinidin (6), malvidin (7) and peonidin (8) as the major anthocyanidins (Aruna *et al.*, 2012). *Aeschynomene grandiflora* has a large number of traditional uses. It contain arginine (9), cysteine (10), histidine (11), isolucine (12), phenylalanine (13), tryptophan, valine (14), threonine (15), alanine (16), asparagine (17), aspartic acid (19) and oleanolic acid (19), (Kirtikar and Basu, 2005).

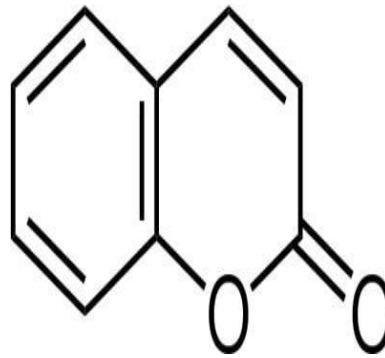
2.4.3 Antimicrobial properties of the *Aeschynomene* species

Antimicrobial activity of the leaf cold water, hot water, methanol and alcohol extracts are more efficient on the selected four pathogens of gram positive (*Bacillus subtilis*, *Staphylococcus aureus*) and gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria than the control drugs Ampicillin and Gentamycin with an average inhibitory zone of 20 mm to 41 mm at 10 mg/well concentration of the drug. Minimum Inhibitory Concentrations (MIC) were observed between 0.55- 0.75 mg/ml equal to that of the control drugs. (Aruna *et al.*, 2012).

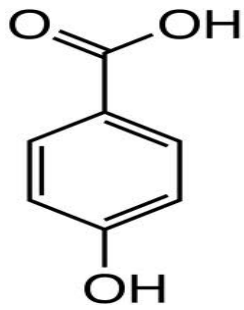
Physicochemical parameters of *Aeschynomene indica* leaf powder were determined and reported as total ash, water soluble ash, acid-insoluble ash, alcohol soluble extractive, water-soluble extractive and moisture content (Anonymous, 1985).



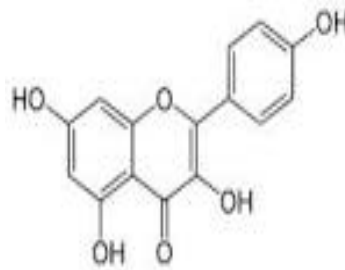
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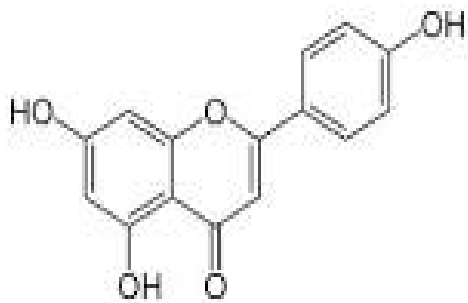
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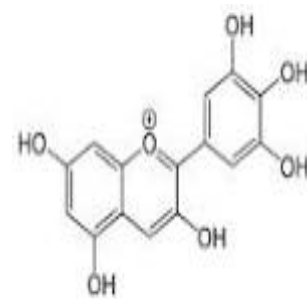
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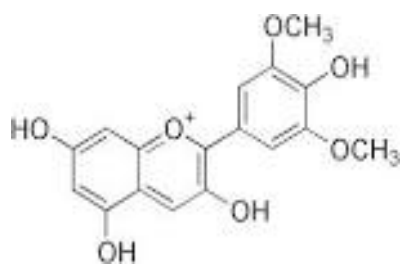
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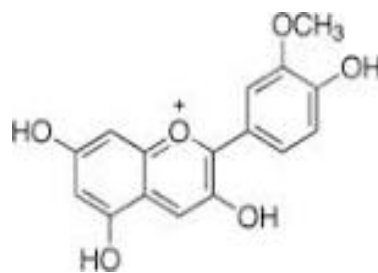
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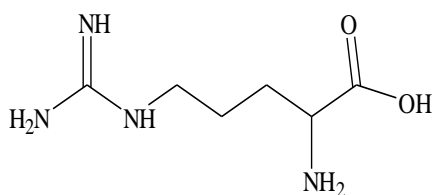
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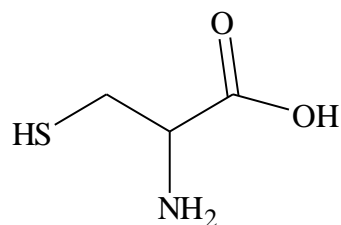
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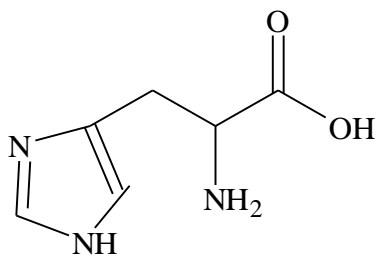
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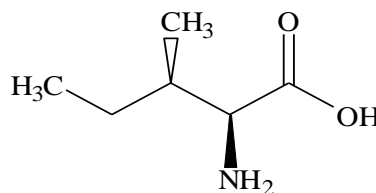
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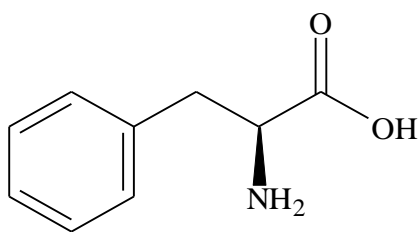
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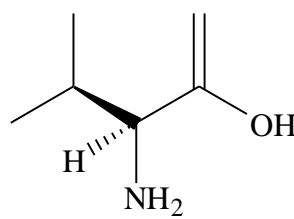
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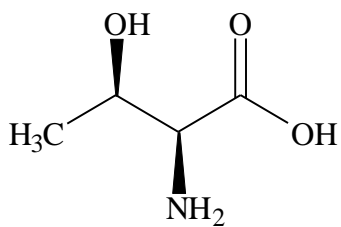
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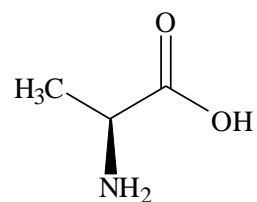
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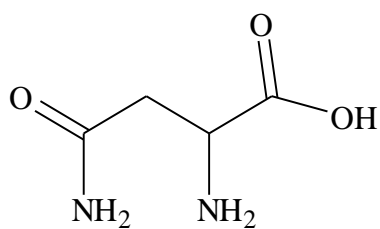
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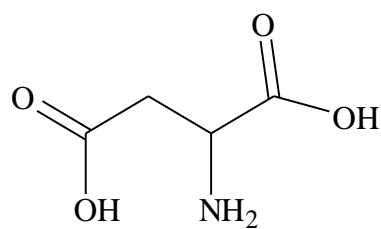
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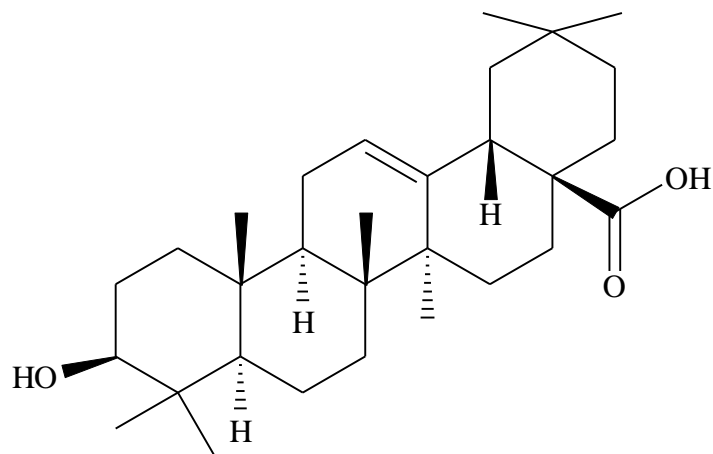
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(17)



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(19)

2.5 The Fabaceae Family

The Fabaceae family, also called Leguminosae or bean and pea family, is the third largest family of angiosperms after Orchidaceae (orchids) and Asteraceae (daisies, sunflowers), and second only to Poaceae (grasses) in terms of agricultural and economic importance (Wojciechowski *et al.*, 2006). The group is the third-largest [land plant](#) family, behind only the [Orchidaceae](#) and [Asteraceae](#), with 750 genera and over 18,000 species (Sprent., 2001). The largest genera are [Astragalus](#) (over 2,400 species), [Acacia](#) (over 950 species), [Indigofera](#) (around 700 species), [Crotalaria](#) (around 700 species), and [Mimosa](#) (around 500 species). Plants of this family are found throughout the world, growing in many different environments and climates. Legumes includes a large number of domesticated species harvested as crops for human and animal consumption as well as for oils, fiber, fuel, fertilizers, timber, medicinals, chemicals, and horticultural varieties (Lewis., 2005). In addition, the family includes several species studied as genetic and genomic model systems (e.g., pea, *Pisum sativum*, barrel medic, *Medicago truncatula*, and trefoil, *Lotus corniculatus*). Morphologically, Fabaceae is characterized by leaves simple to compound (pinnate, rarely palmate, or bipinnate), unifoliate, trifoliate (*Medicago*, *Trifolium*), sometimes phyllodic (many species of *Acacia*), or reduced to a tendril (as in *Lathyrus*), spirally arranged, with stipules present that are sometimes large and leaf-like (*Pisum*) or developed into spines (*Prosopis*, *Robinia*) (Wojciechowski *et al.*, 2006). Flowers are usually regular or irregular (i.e., actinomorphic to zygomorphic in symmetry, respectively), bisexual, with a single superior carpel (hypogynous to perigynous), pentamerous, arranged singly or in racemes, spikes, or heads. The principal unifying feature of the family is the fruit, the legume (Polhill, 1994). With a few exceptions, legumes are typically one-chambered pods (one locule), with parietal placentation along the adaxial suture, in two alternating rows on a single

placenta, typically dry and dehiscent along one or both sutures (legume), occasionally constricted into 1-seeded sections (loment) or indehiscent (samara, drupe, achene). Fabaceae has been traditionally divided into three subfamilies, the Caesalpinioideae, Mimosoideae, and Papilionoideae (although sometimes these have been ranked as separate families, as in *Caesalpinaceae*, *Mimosaceae*, and *Papilionaceae*), and considered most closely related to the Connaraceae and Sapindaceae on the basis of anatomy, morphology, and biogeographic distributions (Polhill *et al.*, 1981). The recognition of three subfamilies is based on characteristics particularly of the flower, including size, symmetry, [aestivation of petals](#), sepals (united or free), stamen number and heteromorphy, pollen (single or polyads), but also presence of a pleurogram, embryo radicle shape, leaf complexity, and presence of root nodules (Lewis *et al.*, 2005). Differences in these characteristics led to the view that the *Mimosoideae* and *Papilionoideae* are unique and distinct lineages in the family which arose independently within a paraphyletic "basal" caesalpinoid assemblage. The Fabaceae contains over 19,000 extant species widely distributed throughout the world in many ecological settings, from deserts of high latitudes to seasonally dry and wet tropical forests of equatorial regions (Lewis *et al.*, 2005). Legumes appear to have diversified early (Herendeen *et al.*, 1992) to become a ubiquitous feature of modern terrestrial biotas, similar to the timing of diversification of several other modern families of angiosperms (e.g., Fabaceae; Manos and Standford, 2001).

The fossil record of the Fabaceae is abundant and diverse, particularly in the tertiary, with fossil flowers, fruits, leaflets, wood, and pollen known from numerous localities; some examples are shown in the figures below (Herendeen *et al.*, 1992). Attempts to estimate the age of legumes and diversification in the family, based on molecular sequence data, have been published in recent years (Wikström *et al.*, 2001).

Legumes have demonstrated agricultural importance for thousands of years, beginning with the domestication of lentils (*Lens esculenta*) in Iran dating to 9,500 to 8,000 years ago, their use as a food source during the prehistory of North and South America (beans, more than 3,000 years ago), and their use by the Roman Empire as a food source and for soil improvement (Graham and Vance, 2003). Today legumes are an increasingly invaluable food source not just for humans, accounting for 27% of the world's primary crop production, but also for farm animals (Graham and Vance, 2003). Legumes were grown on more than 13% of the total arable land under cultivation in the world in 2004 (Gepts *et al.*, 2005). Grain legumes alone contribute 33% of the dietary protein nitrogen needs of humans, while soybeans (*Glycine max*) and peanut (*Arachis hypogaea*) provide more than 35% of the world's processed vegetable oil and a rich source of dietary protein for the poultry and pork industries (Graham and Vance, 2003). While they produce nitrogen-containing protein in abundance, legumes are deficient in sulfur containing amino acids and other nutrients needed by people and animals. For this reason, legumes and cereal crops are often raised together to account for the amino acids and other elements they are each deficient in (Gepts *et al.*, 2005). The primary dietary legumes grown, such as bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), chickpea (*Cicer arietinum*), broad bean (*Vicia faba*), pigeon pea (*Cajanus cajan*), cowpea (*Vigna unguiculata*), and lentils (Graham and Vance, 2003), include representatives of each of the four clades within papilionoids, the genistoids, dalbergioids, Hologalegina, and phaseoloid/millettioids. Many legumes form [root nodules to fix atmospheric nitrogen](#) in a symbiotic relationship with the soil bacteria 'rhizobia'. Legumes are extremely diverse in their abilities to nodulate, not all species can and there is a [wide variety of nodules](#) that form, depending on the species in symbiosis. In addition to their uses as food, legumes are still used as tools in agriculture and forestry as the Romans did (Soltis

et al., 2000). The plants themselves or plant products like leaves and pods can be tilled into the soil as a nitrogen source or legume crops can be rotated with others for soil improvement. These techniques save farmers billions of dollars in the cost of nitrogen fertilizers (Graham and Vance, 2003). Industrially, legumes have many uses in making biodegradable plastics, oils, dyes, and biodiesel fuel. Legumes are used traditionally in folk medicines, but also demonstrate importance in modern medicine. Isoflavones commonly found in legumes are thought to reduce the risk of cancer and lower cholesterol and soybean phytoestrogens are being studied for use in postmenopausal hormone replacement therapy (Graham and Vance, 2003). Legumes also produce a hypoglycemic effect when eaten, making them a recommended food for diabetics (Gepts *et al.*, 2005).

The members of sub family *Papilionaceae* are herbs, shrubs or trees found in all climates but mostly between and near the tropics and are more abundant in the old than in the New World. The family includes the greatest number of Legumes, comprising 400 genera with about 7000 species. It is an extremely important family and its members yield nutritious food, fiber, shelter, valuable medicines and also virulent poisons (Datta and Mukherji, 1952). The members exhibit most varied properties, some are amylaceous, other oleaginous, many yield resins, balsams and dyes, a few are astringent, acrid and bitter, narcotic and poisonous, emetic and purging, tonic and restorative . The seeds are often anti periodic and the root anthelmentic. Some of the important genera are *Abrus sp.*, *Alhagi sp.*, *Arachis sp.*, *Butea sp.*, *Cajanus sp.*, *Cicer sp.*, *Derris sp.*, *Glycine sp.*, *Glycyrrhiza sp.*, *Medicago sp.*, *Pisum sp.*, *Phaselous sp.*, *Psoralea sp.*, *Sesbania sp.*, *Tephrosia sp.*, *Vicia sp.* and *Vigna sp.*

2.5.1 Phytochemical constituents

The preliminary phytochemical screening of the three extracts of *Indigofera linnaei* (pet. Ether, chloroform and methanol) revealed the presence of alkaloids, saponins, flavonoids, cardiac glycoside, steroids and tannins (Sandhyavali *et al.*, 2012). Preliminary phytochemical test of the methanol extract of *Pongamia pinnata* leaves (*fabaceae*) reveals the presence of steroids, glycosides, carbohydrates, flavonoids, tannins and saponins (Anupriya *et al.*, 2011). Generally, the different solvent extracts of *Samanea saman* (*fabaceae* or *mimosaceae*) pods indicated low to high presence of alkaloids, saponins and resins but absence of acidic compounds (Obasi *et al.*, 2010). The results indicated that flavonoids were moderately present in ethyl acetate extract but absent in distill water extract, methanol extract and ethanol whereas glycosides were absent in distill water extract but present in methanol extract and ethanol extract in moderate abundance and in ethyl acetate extract in high abundance. Tannins were absent in all the tested extracts but present in methanol extract in moderate abundance while steroids and terpenoids were absent in distill water extract and ethyl acetate extract but present in methanol extract and ethanol extract in varying abundance (Obasi *et al.*, 2010). The methanol soluble part of *Lotus garcinii*, belonging to the family *Fabaceae*, yielded three new metabolites: garceine, garoside (20) and garthiol (21), which have never been detected from any natural source. In addition, isophytol (22), hexadecanoic acid (23), cholesterol (24), oleanolic acid (19), butulinic acid(25) and lupeol (26) were also obtained for the first time from *L. garcinii* (Muhammad *et al.*, 2001).

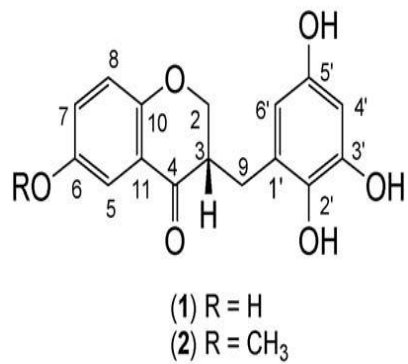
Previous chemical studies on *C.bonduc* have resulted in the isolation of cassane furanoditerpenes and flavonoids (Peter *et al.*, 1997). Investigations on the methanolic extract of *C.bonduc* afforded two new homoisoflavonoids, caesalpinianone (20) and 6-Omethyl

caesalpinianone (27) along with five known natural products, namely, hematoxylol, stereochoenol A, 6-O-acetylloganic acid, 4-O-acetylloganic acid, and 2-O-b-D-glucosyloxy-4-methoxybenzenepropanoic acid (Athar *et al.*, 2009). (3 β)(4-hydroxy-E-cinnamoyl) olean-5,12-diene-28-ol (28) and (3 β)-(2,3,4-trihydroxy-z-cinnamoyl)olean-5-ene-12,28-diol (29) was reported to be isolated from the methanol fraction of *Clotalaria ncana* (Fabaceae) (Zafrul *et al.*, 2013). β -stigmasterol (30) and β - sitosterol (31) were also isolated from the petroleum ether soluble fraction of crude methanol extracts of leaves of *Clotalaria ncana* (Fabaceae) by combination of column chromatography and preparative thin layer chromatography (Zafrul *et al.*, 2013).

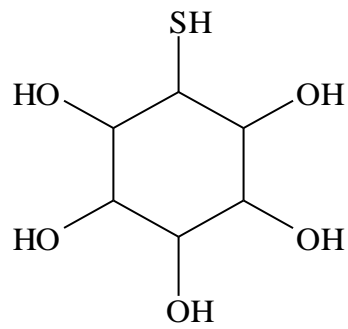
Triterpenoids saponins (e.g) glycyrrhizin (32) and glabranin (33) have been isolated from *Glycyrrhyza glabra* (Linn) Papilionaceae/ Fabaceae. *Glycyrrhizic* acid (34) isolated from this *glycyrrhyza glabra linn papilionaceae/ fabaceae*, protect against aflotoxins (Pandy Govind 2011). β -amyrin (35) stigmasterol (30), isoliquiritin (36) and liquiritoside (37) have also been isolated from *glycyrrhyza glabra* (Linn) Papilionaceae/Fabaceae (Pandy Govind, 2011). Bavachinin (38), corylfolynini and psoralen (39), isolated from *Psoralea corylifolia* possess strong anti-cancer activity against lung cancer and liver cancer (Pandy Govind 2011).

The result of the phytochemical screening of the methanol extract and two solvent fractions of *indegofera conferta* GILLETT (Papilionaceae) revealed the presence of flavonoids and tannins while, steroid were only detected in the methanol extract (Musa *et al.*, 2008). The presence of tritermen, sterole (40), eugenole (41), indole (42), and glycoside (43) have been reported in *Glycyrrhyza glabra* (Linn) Papilionaceae/ Fabaceae by Pandy *et al.*, (2011). The preliminary phytochemical screening of *Crotolaria lachnosema* Stapf. (Papilionaceae) revealed the presence of saponins, terpenes, sterols, flavonoids, resins and balsams while tannins,

cardiac glycosides, alkaloids, phlobatannins and glycosides were absent (Jemilat *et al.*, 2012). Preliminary phytochemical screening of *Pongamia Pinnata* (*papilionaceae*) extracts showed the presence of alkaloids, carbohydrates, fats and resins (Kumar *et al.*, 2013).

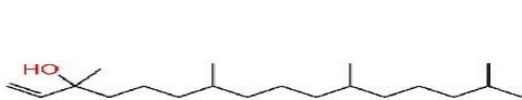


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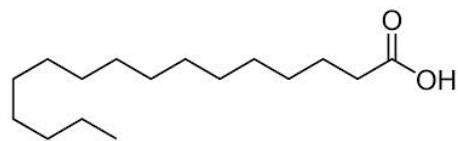


2,3,4,5,6-pentahydroxy cyclohexathiol (garthiol)

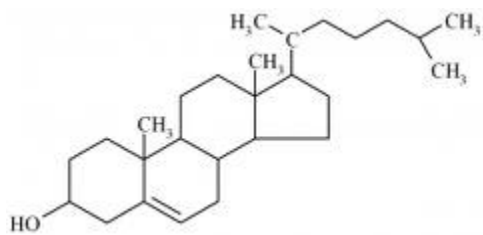
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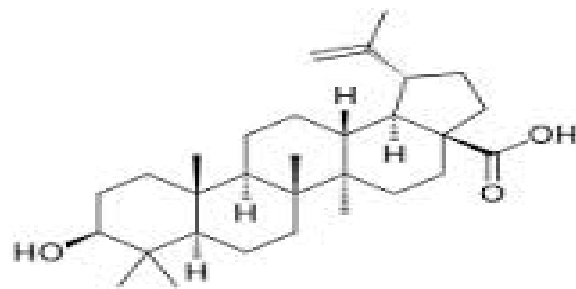
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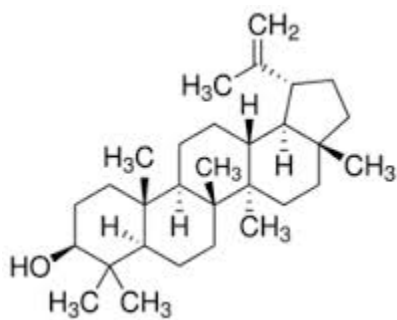
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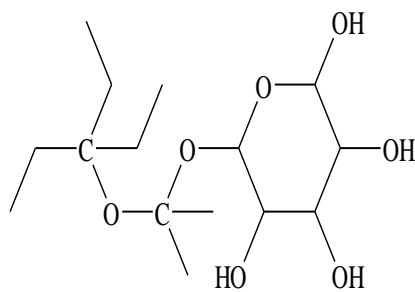
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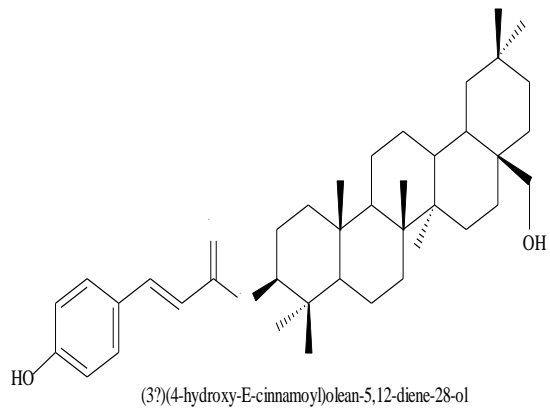
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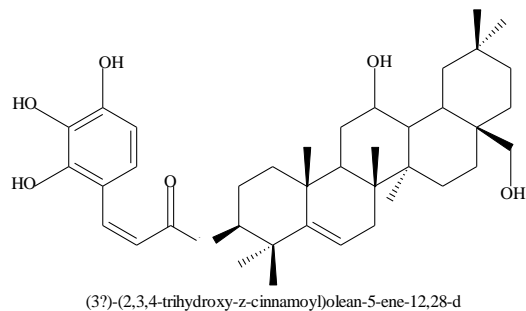
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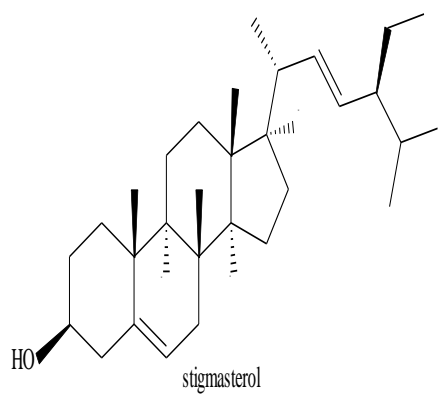
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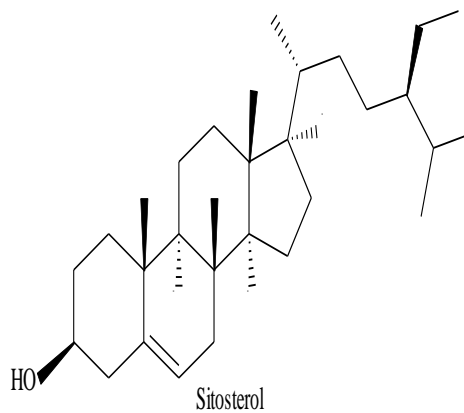
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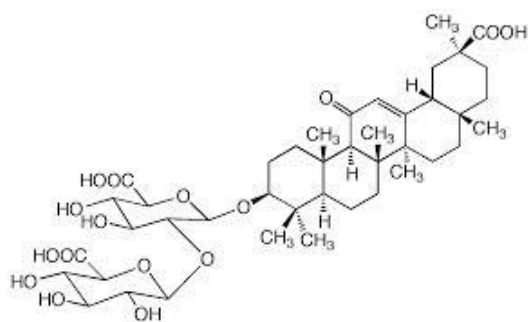
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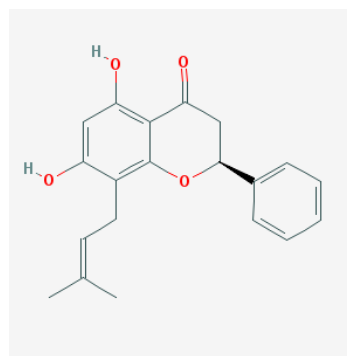
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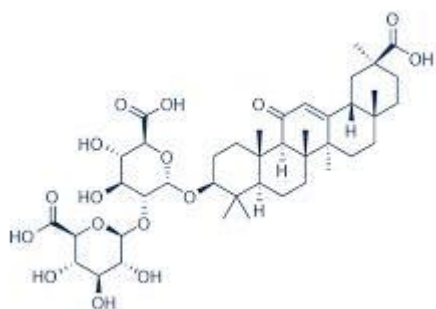
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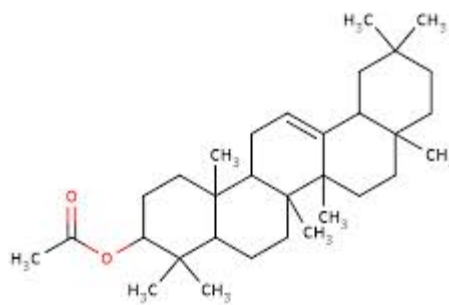
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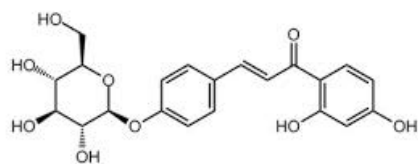
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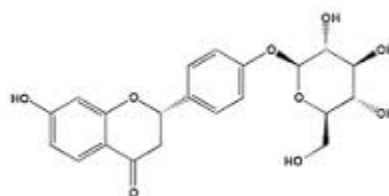
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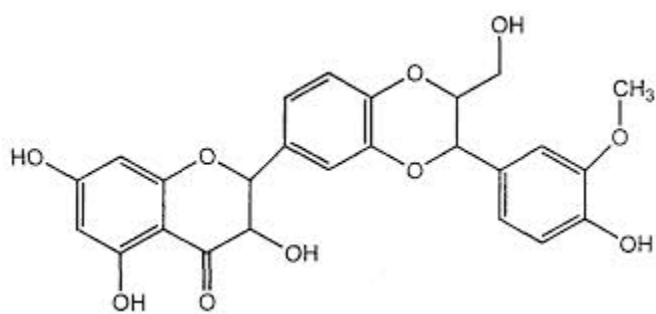
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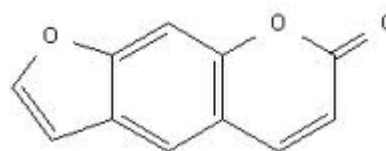
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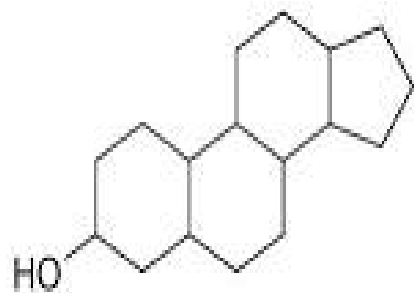
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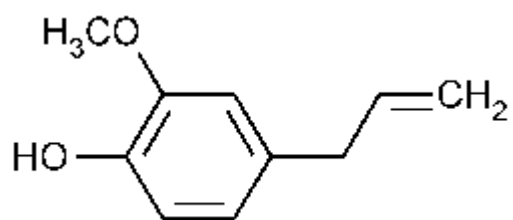
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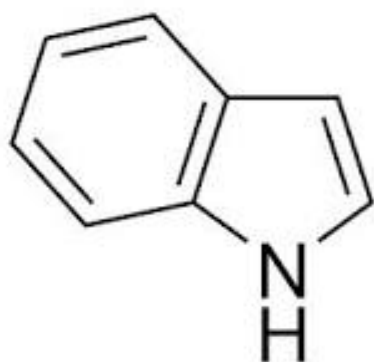
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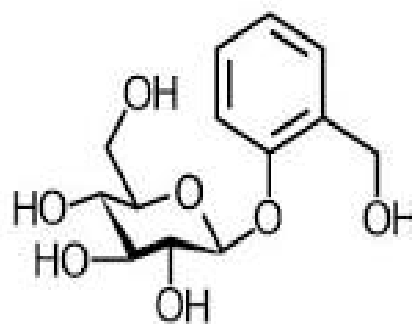
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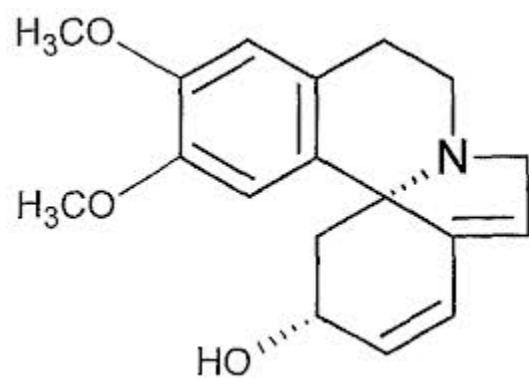
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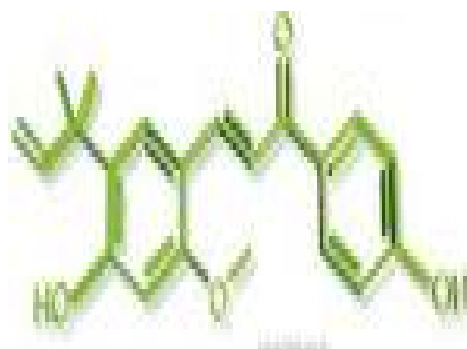
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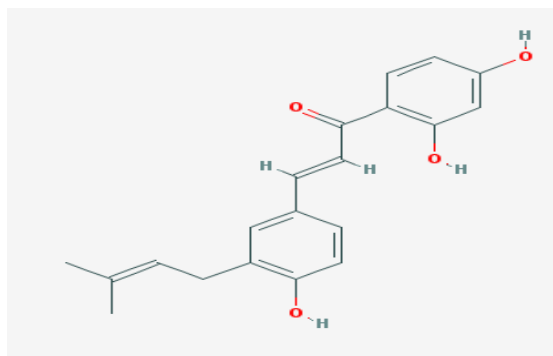
(43)



(44)



(45)



(46)

2.5.2 Antidiabetic activity

The aqueous and ethanolic extracts of the seeds of *Caesalpinia bonduc* L. (synonymous name: *C. bonducella*; Family: *Fabaceae*) have been reported to possess in vivo hyperglycemic effect in alloxan and streptozotocin induced type 2 diabetes in a rat model at a dose of 25 mg/kg body weight (Chakrabarti *et al.*, 2003).

2.5.3 Antimicrobial activity

The methanolic, ethyl acetate and water fractions of crude extracts exhibit in vitro activity against the growth of an array of pathogenic bacteria and fungi (Simin *et al.*, 2001). Methanolic extract from *Indigofera linnaei* was also screened for antifungal activity using Itraconazole as standard. Among the two fungi, extract showed moderate activity against *Candida tropicalis* and mild activity against *Candida balanitis* (Sandhyavali *et al.*, 2012). The antimicrobial activity of *Samanea saman* (*fabaceae* or *mimosaceae*) pods of different solvent extracts revealed that methanol extract inhibited two bacteria species (*B.subtilis* and *S. aureus*) whereas ethyl acetate extract inhibited only *S.aureus* (Obasi *et al.*, 2010).

2.5.4 Anticonvulsant activity

Flausino *et al.*, (2007), reported that both anxiolytic alkaloids (+)-erythravine and (+)-11- α -hydroxy-erythravine (44) isolated from the flowers of *Erythrina mulungu* Mart ex Benth

(Leguminosae–Papilionaceae) exhibited significant anticonvulsant activity. The hydroalcoholic extract of the stem bark from specimens of *E. mulungu* inhibits seizures induced in mice by the administration of strychnine and PTZ. These alkaloids promote anxiolytic effects when administered to mice.

2.5.5 Antibacterial activity

The methanolic extract from was screened for antibacterial activity using streptomycin as standard. Among the four strains of the bacteria, the extract showed considerable activity against *Bacillus subtilis* and moderate activity in case of *Staphylococcus aureus*. However, very mild activity was observed against gram-ve organisms i.e. *Escheria coli* and *Klebsiella pneumoniae*. (Sandhyavali *et al.*, 2012).

2.5.6 Anticancer activity

The methanol extract of *Crotalaria ncana* (Fabaceae) revealed the antiproliferative activity against the HEP-2(laryngeal cancer) and NCI-H292 (lung cancer) cell lines using the (3-[4, 5-dimethyl thiazol-2-yl]-2, 5-diphenyltetrazole) (MTT) method (De MELO *et al.*, 2010).

2.5.7 Antioxidant activity

Crotalaria ncana (Fabaceae) possesses a significant antioxidant activity which was evaluated with DPPH (2, 2-diphenyl-2-picrylhydrazyl) (De Melo *et al.*, 2001). In the *in vitro* antioxidant study which was carried out by DPPH assay method, methanolic extract showed good antioxidant activity with IC₅₀ values of 50 µg/ml, 100 µg/ml, 250 µg/ml, 500 µg/ml and 1000 µg/ml as compared to the chloroform extract which showed less inhibitory effect but in case of hydrogen peroxide assay chloroform extract showed good antioxidant activity compared to methanol extract. (Sandhyavali *et al.*, 2012). The methanolic extract of *F. strobilifera* root and

leaf possesses good antioxidant activity, which might be helpful in preventing the progress of various oxidative stresses (Madan *et al.*, 2010).

2.5.8 Antimicrobial activity

The crude methanol extract and residual water fraction of *Indigofera conferta* GILLETT (Papilionaceae) were found to have MIC values of 4-6 mg/ml and MBC at 5-7 mg/ml while the n-butanol fraction had MIC values ranging from 5-6 mg/ml and MBC at 6-7 mg/ml (Musa *et al.*, 2008).

2.5.9 Anti-cancer activity

Flavonoids derived from the root, rhizome or whole plant of *Glycyrrhiza glabra* linn Papilionaceae/ Fabaceae possessed strong anticancer activity. Licochalchone A (45). inhibits growth and spreads of various cancer cells particularly the androgen refractory prostate cancer by inducing apoptosis and arresting cancer cell division. Licoagrochalcone (46) possesses strong anticancer activity against cancer of the breast, lungs, stomach, colon, liver and kidney (Pandy Govind 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Reagents

The solvents used were all of analytical grade and were all distilled at suitable temperatures after use. The solvents were of the JHD brand and purchased from ROMPTECH Chemical Enterprise Danmagaji, Zaria. They are Petroleum ether (60-80 °C), Chloroform, Ethyl acetate and Methanol.

3.1.2 Equipments

- (i). NMR spectra were obtained on a Bruker AVANCE spectrometer (400 MHz for ^1H and 125 MHz for ^{13}C), using the TMS peaks as standard.
- (ii). FTIR Spectroscopy (Shimadzu FTIR8 400s Fourier Transform Infra-Red Spectroscopy).
- (iii). The melting point of the compound was determined using Ernst Leitz Wetzlar melting point.

3.1.3 Collection and identification of plant materials

The whole plant of *Aeschynomene uniflora* was collected fresh from Benue State, Nigeria in September, 2012. Taxonomical identification was done at the Herbarium of the Biological Sciences Department, A.B.U, Zaria, Nigeria and its voucher specimen with number 2408 deposited there. The plant was air-dried under shade, segregated and pulverized by mechanical

pounding using wooden mortar and pestle. The pulverized plant material was stored away from moisture.

3.1.4 Microbiological media

i. Muller Hinton agar

ii. Sabouraud dextrose agar

3.2 Methods

3.2.1 Extraction

The pulverized plant material (500 g) was carefully weighed and packed into a soxhlet extractor and successively extracted with petroleum ether (60-80°C), chloroform, ethyl acetate and followed by methanol and each was exhaustive.

The extracts were concentrated in vacuum at 40°C using rotator evaporator and later subjected to air drying to give dried crude extracts. The extracts were weighed and their weights recorded.

3.2.2 Phytochemical screening

The petroleum ether, Chloroform, ethyl acetate and the methanol extracts were subjected to phytochemical screening using standard techniques (Harborne 1973). The metabolites tested for included, carbohydrates, tannins, saponins, flavonoids, anthraquinones, cardiac glycosides, steroids, terpenes and alkaloids.

3.2.2.1 Test for carbohydrates (molischs test)

The four crude extracts (1g respectively) were dissolved in about 5 ml of distilled water and heated in a water bath. The solution was filtered. To the filtrate, four drops of molischs was added. 3 ml of concentrated sulphuric acid was carefully added to the mixture from the side of the test tube to form a lower layer. A purple color appeared at the interface on all the extracts.

3.2.2.2 Test for tannins (ferric chloride test)

The four crude extracts were respectively dissolved in 10 ml of the distilled water and shaken vigorously for about 30 seconds. It was allowed to stand. A honey comb like structure was observed in the ethyl acetate which lasted for more than 30 minutes.

3.2.2.3 Tests for flavonoids (ammonical silver nitrate test)

The four crude extracts (0.5 g) were respectively dissolved in 5ml of distilled water. Four drops of the solution of ammonical silver nitrate were added. The ethyl acetate and the methanol extracts gave a yellowish – brown color while the other two showed no coloration. On heating it was observed that the color turned brownish black.

3.2.2.4 Test for anthraquinones. (Bronstrager's test)

The four extracts (0.5 g) were respectively dissolved in 10 ml of chloroform and shook properly and filtered. 5 ml of 10% of ammonia solution was added to the filtrate and stirred. There was no coloration observed in any of the extracts.

3.2.2.5 Test for cardiac glycoside (kella-killani test)

The four crude extract (0.5 g) were respectively dissolved in 5 ml glacial acetic acid containing traces of ferric chloride in a test tube was held at an angle of 45° and 1 ml of concentrated sulphuric acid was added carefully down the side. All the four samples showed purple ring colour at the interface.

3.2.2.6 Test for steroids and triterpenes (leiberman-burchards test)

Acetic anhydride (5 ml) was respectively added to 5 ml of each of the four extracts in a test tube. 1 ml of concentrated sulphuric acid was added carefully down the side of the test tube. A pink colour appeared in the chloroform, ethyl acetate and methanolic extracts which later changed into a blue green colour. The petroleum ether extracts showed no coloration

3.2.2.7 Test for alkaloids

Few drops of Mayer's reagent were added to about 0.5 g each of the four crude extracts in a test tube. There were was no precipitate

3.2.3 Antimicrobial Screening

Antimicrobial activities of the petroleum ether, chloroform, ethyl acetate and methanolic extracts were determined using some pathogens.

3.2.3.1 Collection and preparation of microbial culture;

The pure clinical bacteria and fungi isolates of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium ulcerans*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Candida albicans*, *Candida stellatoidea*

and *Candida krrusei* were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria. All the microbes were screened for purity and were maintained in slants of nutrient agar for bacteria and slants of sabouroud dextrose agar for fungi.

3.2.3.2 Preparation of solution of plant extracts for antimicrobial screening test

Well diffusion method was used for the determination of the antimicrobial activities of the extracts. Each crude extracts was weighed out and dissolved in 10 ml of Dimethylsulphoxide (DMSO) to obtain a concentration of 10 mg/ml. This was the initial concentration used to check the antimicrobial activities of the extracts.

3.2.3.3 Media preparation

Muller Hinton and Sabouroud dextrose agar were the media used as the growth media. They were prepared according to the manufacturer's instruction, boiled to dissolved and were sterilized at 121°C for 15 minutes. The media were cooled to 45°C and 29 ml of the sterilized media were poured into sterilized petri dishes. The media were allowed to cool and solidify and then cooled.

3.2.3.4 Determination of microbial activities of the various extracts

The media prepared above were inoculated with 0.1 ml standard inoculums of the test organisms. The inoculums were spread evenly over the surface of the medium by the use of a sterile swab. The agar plates were seeded with the test organisms and the sabouroud dextrose agar with the fungi. The inoculated plates were incubated at 37 °C for 24 hrs for the bacteria and at 30 °C for 1 – 7 days for the fungi. Standard cork borer of 6 mm diameter was used to cut a well at the centre of each inoculated plate and 0.1 ml of the solution of the extracts was introduced into each

well. The plates were then incubated at 37°C for 24 hours for the bacteria and at 25°C for 24 hours for the fungi. They were observed after the periods of incubation for zone of inhibition of growth. The zones were measured with a caliper and the values were recorded in millimeters.

3.2.3.5 Determination of minimum inhibitory concentrations (MIC) of the extracts

The minimum inhibitory concentration of the extracts were carried out on the test organisms using the broth dilution method. Nutrient broth was prepared according to the manufacturer's instructions. Mc-farlands turbidity scale number 0.5 was prepared to give turbid solution. Normal saline was prepared and was dispensed into test tubes and the microorganisms were then inoculated and incubated at 37 °C for 6 hours. Dilution of the test microorganisms in the normal saline was performed until the turbidity matched that of the Mac farlands scale by visual comparison. At this point the microorganisms had a concentration of about 1.5×10^8 cfu/ml. Two fold serial dilutions of the extracts in the broth were performed to obtain the concentration of 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml and 0.625 ml respectively. The Initial concentration was obtained by dissolving 0.1 g of the extracts in 100 ml of the sterile broth. Having obtained the different concentrations of the extract in the broth, 0.1 ml of the standard inoculum of the test microorganism in the normal saline was then inoculated in to the different concentrations. Incubation was made at 37 °C for 24 hrs after which each broth was observed for turbidity. The lowest concentration of the extract in the broth which showed no turbidity was recorded as the Minimum Inhibition Concentration (MIC).

3.2.3.6 Minimum Bactericidal Concentration/ Minimum Fungicidal Concentration

The minimum bactericidal concentration/minimum fungicidal concentrations (MBC/MFC) were carried out to determine if the test microbes were killed or only their growth was inhibited. Mueller-Hinton agar was prepared and sterilized at 121 °C for 15 minutes, poured into Petri dishes and were allowed to cool and solidify. The content of the MIC in the serial dilution was sub - cultured onto the prepared medium and incubation was done at 37 °C for 24 h. Thereafter each plate of the medium was observed for colony growth. The value obtained in the plate with lowest concentration of the extracts without colony growth was recorded as the MBC/MFC (Bauer *et al.*, 1977).

3.2.4 Chromatographic Purification of Extracts

3.2.4.1 Thin layer chromatography (TLC)

Thin layer chromatography (TLC) was performed on the four extracts to ascertain the number of components present in them. This test was carried out using precoated silica gel analytical TLC plates prepared by MERCK. The previous extracts were separately dissolved in minimum volume of their respective extracting solvent. The extracts were spotted on TLC plates and allowed to dry. The plates were developed in a development tank using perceived solvent mixture that would give a H_2SO_4 and heated in an oven for about 1 minute.

3.2.4.2 Spotting and development

The precoated TLC plate were manually spotted using capillary tube, the plates were dried and developed in an air-tight chromatographic tank at room.

3.2.4.3 Visualization of spots

Spots on the precoated TLC plates were visualized under UV light (254-366nm) and spraying with 10 % sulphuric acid, followed by heating at 110 °C for 5-10 min.

3.2.4.4. Purification and Isolation of compounds

A small portion of Petroleum Ether fraction (PE) of hot and cold extraction was dissolved in petroleum ether and the solution was spotted on TLC plates. Then the TLC plates were run by specific solvent systems and were viewed individually under UV light and also with the vanillin-H₂SO₄ reagent (Bobelt, 1963). Through several pilot experiments, it was found that the constituent of petroleum ether fraction were separated by the solvent system of n-Hexane and ethyl acetate in the proportion of 9:1. The petroleum ether fraction, 10 g, was subjected to column chromatography on a silica gel (60-120 mesh) stationary phase with gradient elution using n-hexane : ethyl acetate and finally with 100% methanol (Srivastave and Srivastave, 1987). Two fractions were found homogeneous on TLC plate by using n-hexane: ethyl acetate (9:1), n-hexane: chloroform (10:1), petroleum ether: ethyl acetate (9:1) and petroleum ether : methanol (7:3) solvent systems.

3.2.4.6 Preparative thin layer chromatography

The purified sample from the above column was subjected to preparative TLC. The different layers were scraped out and recovered using chloroform. The result gave rise to a component named AEPE.

CHAPTER FOUR

4.0 RESULTS

4.1 Result of phytochemical screening

The preliminary phytochemical screening of the aerial part of *Aeschynomene uniflora* showed the presence of carbohydrates, in all the four extracts, Cardiac glycosides were present in all the extracts except in petroleum ether extracts. Carbohydrates, cardiac glycoside, tannins, flavonoids, steroids and triterpenes were present in the ethyl acetate, chloroform and methanol extracts. Saponins were present only in the methanol extract, while alkaloids and anthraquinones were absent in all the four extracts (Table 4.1).

Table 4.1: Phytochemical screening of the extracts of *Aeschynomene uniflora*

Metabolites	PE	CH	ET	ME
Carbohydrate	+	+	+	+
Cardiac glycoside	-	+	+	+
Tannins	-	-	+	+
Saponins	-	-	-	+
Flavonoids	-	-	+	+
Anthraquinones	-	-	-	-
Steroids	+	+	+	+
Triterpenes	+	+	+	+
Alkaloids	-	-	-	-

Key: + = present, - = absent, PE = Petroleum ether extract, CH = Chloroform extracts, ET = Ethyl acetate extracts, ME = Methanol extracts

4.2 Result of Anti-Microbial Screening of Extracts

From the results of the antimicrobial screening (Tables 4.2), the extracts showed activity against *S. pyogenes*, *B. subtilis*, *C. stellatoidea*, *K. pneumoniae*, *C. albicans* and *S. aureus*. The petroleum ether extract was effective against *S. pyogenes*, *B. subtilis*, *S. stellatoidea*, *K. pneumoniae*, *S. aureus*, *C. albicans* and with a zone of inhibition of -16, -17, -17, -18, -18 and -18 mm respectively (Table 4.3). The minimum inhibitory concentration (MIC) showed that the petroleum ether extracts inhibited the growth of all the pathogenic microorganisms at the concentration of 30 mg/ml. The minimum bactericidal/fungicidal concentration (MBC/MFC) was found to be 60 mg/ml against the entire test micro organism (Table 4.4). The chloroform extracts showed activity against *S. aureus*, *S. pyogenes*, *B. subtilis*, *K. pneumoniae*, *C. albicans* and *C. stellatoidea* with zone of inhibition of -22, -22, -23, -24, -24 and -27 mm, respectively (Table 4.3). At the MIC of 15 mg/ml, the chloroform extract inhibited the growth of *S. aureus*, *S. pyogenes*, *K. pneumoniae*, *C. albicans* and *C. stallatoidea*; while at 7.5 mg/ml the growth of *B. subtilis* was inhibited. The MBC/MFC for the chloroform extract was 30 mg/ml, and at this concentration, the extract exhibited activity against all the test microorganism except *S. pyogenes* which had 60 mg/ml (Table 4.4). The MIC showed that at lower concentration of 7.5 mg/ml the ethyl acetate extract can inhibit the growth of *B. subtilis* and *C. stellatoidea*, but the inhibition of *S. aureus*, *S. pyogenes*, *B. subtilis*, *K. pneumoniae* and *C. albicans* occurred at 15 mg/ml. The MBC/MBF (30 mg/ml) of ethyl acetate extract was active against *S. aureus*, *B. subtilis*, *K. pneumoniae*, *C. albicans* and *C. stallatoidea* and the value for *S. pyogenes* was 60 mg/ml. The methanol extract induced significant zone of inhibition for *C. stallatoidea*, *C. albicans*, *S. pyogenes*, *S. aureus*, *B. subtilis*, and *K. pneumoniae* with values -19, -20, -20, -21 and -22 mg/ml respectively (Table 4.3). At MIC of 15 mg/ml for *S. aureus*, *S. pyogenes*, *B. subtilis*, *K.*

pneumoniae, and *C. albicans*. At MIC of 30 mg/ml the growth of *C. stallatoidea* was inhibited, while other microbes were inhibited at the values of 15 mg/ml. At an MBC/MFC of 60 mg/ml the methanol extract exhibited activity against all the microbes; with the exception of *B. subtilis*, inhibited at 30 mg/ml (Table 4.4). Among the four extract tested, the ethyl acetate extract demonstrated the highest activity against all the microorganism.

Table 4.2: Antimicrobial Sensitivity test of the various extracts against test organisms

Test organism	PE	CH	ET	ME	Ciprofloxacin	Fluconazole
<i>Staphylococcus aureus</i>	S	S	S	S	S	R
<i>Streptococcus pyogenes</i>	S	S	S	S	S	R
<i>Corynebacterium ulcerans</i>	R	R	R	R	S	R
<i>Bacillus subtilis</i>	S	S	S	S	S	R
<i>Escherichia coli</i>	R	R	R	R	S	R
<i>Klebsiella pneumonia</i>	S	S	S	S	S	R
<i>Proteus mirabilis</i>	R	R	R	R	R	R
<i>Pseudomonas aeruginosa</i>	R	R	R	R	R	R
<i>Proteus vulgaris</i>	R	R	R	R	S	R
<i>Candida albicans</i>	S	S	S	S	R	S
<i>Candida stellatoidea</i>	S	S	S	S	R	S
<i>Candida krusei</i>	R	R	R	R	R	S

Key: PE = Petroleum ether extract, CH = Chloroform extracts, AU = Ethyl acetate extracts, ME = Methanol extracts **R = Not Sensitive** **S = Sensitive**

Table 4.3 Determination of Zones of Inhibitory (mm) of the extracts on test organisms

Test organism	PE	CH	ET	ME	Ciprofloxacin	Fluconazole
<i>Staphylococcus aureus</i>	18	24	25	21	35	Nil
<i>Streptococcus pyogenes</i>	16	22	22	20	30	Nil
<i>Bacillus subtilis</i>	17	27	28	22	42	Nil
<i>Klebsiella pneumonia</i>	18	24	23	20	44	Nil
<i>Candidas albicans</i>	18	22	24	20	Nil	40
<i>Candida stellatoidea</i>	17	23	26	19	Nil	32

Key: PE = Petroleum ether extract, CH = Chloroform extracts, AU = Ethyl acetate extracts, ME = Methanol extracts

Table: 4.4 Minimum Inhibitory Concentration/Minimum Bactericidal Concentration (mg/ml)

Test organism	MIC				MBC/MFC			
	PE	CH	ET	ME	PE	CH	ET	ME
<i>Staphylococcus aureus</i>	30	15	15	15	60	30	30	60
<i>Streptococcus pyogenes</i>	30	15	15	15	60	60	60	60
<i>Bacillus subtilis</i>	30	7.5	7.5	15	60	30	30	30
<i>Klebsiella pneumonia</i>	30	15	15	15	60	30	30	60
<i>Candida albicans</i>	30	15	15	15	60	30	30	60
<i>Candida stellatoidea</i>	30	15	7.5	30	60	30	30	60

Key: PE = Petroleum ether extract, CH = Chloroform extracts, ET = Ethyl acetate extracts, ME = Methanol extracts

4.3 Result of Antimicrobial Screening of AEPE

Antimicrobial tests were carried out to evaluate antimicrobial activities of the isolated compounds using agar diffusion method and ten microorganisms species: *C. albicans*, *C. stellatoidea*, *C. krusei*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *S. aureus*, and *S. pyogenes* (Table 4.5). The activities of the compounds were expressed in terms of growth inhibition zones (given in mm). The growth inhibitory activity of the compound is given in (Table 4.6).

Similar to that of the crude extracts, the antibacterial activities of the isolated compounds were lower than that of the reference drug (ciprofloxacin and Fluconazole) against all bacterial species used in the experiment, and their growth inhibition values were also comparable to each other.

The isolated compound AEPE was effective against: *C. stellatoidea*, *E.coli*, *C. albicans*, MRSA, *S.aureus*, *S. pyogene*, *P.mirabilis*, *C.krusei* and *K. pneumoniae* with a zone of inhibition of -25, -25, -27, -28, -30, -31 and -34 mm respectively (Table 4.6). The minimum inhibitory concentration (MIC) showed that AEPE inhibited the growth of *S. aureus*, *P. mirabilis* and *K. pneumoniae* at the concentration of 3.2 µg/ml and at a concentration of 7.5 µg/ml, the growth of *C. stellatoidea*, *E.coli*, and *C.albicans* were inhibited (Table 4.7).

The minimum bactericidal/fungicidal concentrations (MBC/MFC) was found to be 7.5 mg/ml against *S.aureus*, *P.mirabilis* and *K. pneumonia*, while the other microbes were inhibited at the value of 15 mg/ml (Table 4.7).

Table 4.5 Antimicrobial activities of isolated compound AEPE

Test organism	AEPE	Ciprofloxacin	Fluconazole
<i>Candida albicans</i>	S	R	S
<i>Candida stellatoidea</i>	S	R	S
<i>Candida krusei</i>	R	R	S
<i>Escherichia coli</i>	S	S	R
<i>Klebsiella pneumonia</i>	S	S	R
<i>Pseudomonas aeruginosa</i>	R	S	R
<i>Proteus mirabilis</i>	S	S	R
<i>Staphylococcus aureus</i>	S	S	R
<i>Streptococcus pyogenes</i>	R	S	R

Table 4.6 Zones of inhibition of AEPE against the test microorganism (mm)

Test organism	AEPE	Ciprofloxacin	Fluconazole
<i>Candida albicans</i> ,	27	0	35
<i>Candida stellatoidea</i>	25	0	32
<i>Escherichia coli</i>	25	35	0
<i>Klebsiella pneumonia</i>	34	40	0
<i>Proteus mirabilis</i>	31	30	0
<i>Staphylococcus aureus</i>	25	35	0

Table 4.7 Minimum Bactericidal Concentration/ Minimum Fungicidal Concentration (µg/ml)

Test organism	MIC AEPE	MBC/MFC AEPE
<i>Staphylococcus aureus</i>	3.25	7.5
<i>Escherichia coli</i>	3.25	7.5
<i>Candida albicans</i>	7.5	15
<i>Candida stellatoidea</i>	7.5	15
<i>Escherichia coli</i>	7.5	15
<i>Proteus mirabilis</i>	3.25	7.5

4.4 Result of FTIR spectrum of AEPE

The IR spectrum of AEPE, showed a very intensely broad absorption peak at 3416cm^{-1} typical of O-H bond stretch of hydroxyl group. A moderate intense band at 2933 cm^{-1} can be assigned to aliphatic C-H stretch and at 2359.39 cm^{-1} a weak C-O Stretch is observed (Figure 1). The C = C vibrations was observed at 1732.13 cm^{-1} as an intense band and a corresponding out of plane C-H vibrations of the unsaturated part was observed at 1062.81 cm^{-1} (Table 4.8).

Table: 4.8 Infra Red Table

Serial no.	Frequency, cm^{-1}	Bond	Functional group
1	1062.81	C-H	Alkenes
2	1381.08	C-Os	Alcohols,
3	1464.02	C-H bend	Alkanes
4	1732.13	C=C	Alkenes
5	2359.02	C-O sharp stretch	Alcohols
6	2864.39	C-H sharp stretch	Alkanes
7	2933.83	C-H stretch	Alkanes
8	3416.05	O-H stretch, H-bonded	Alcohols

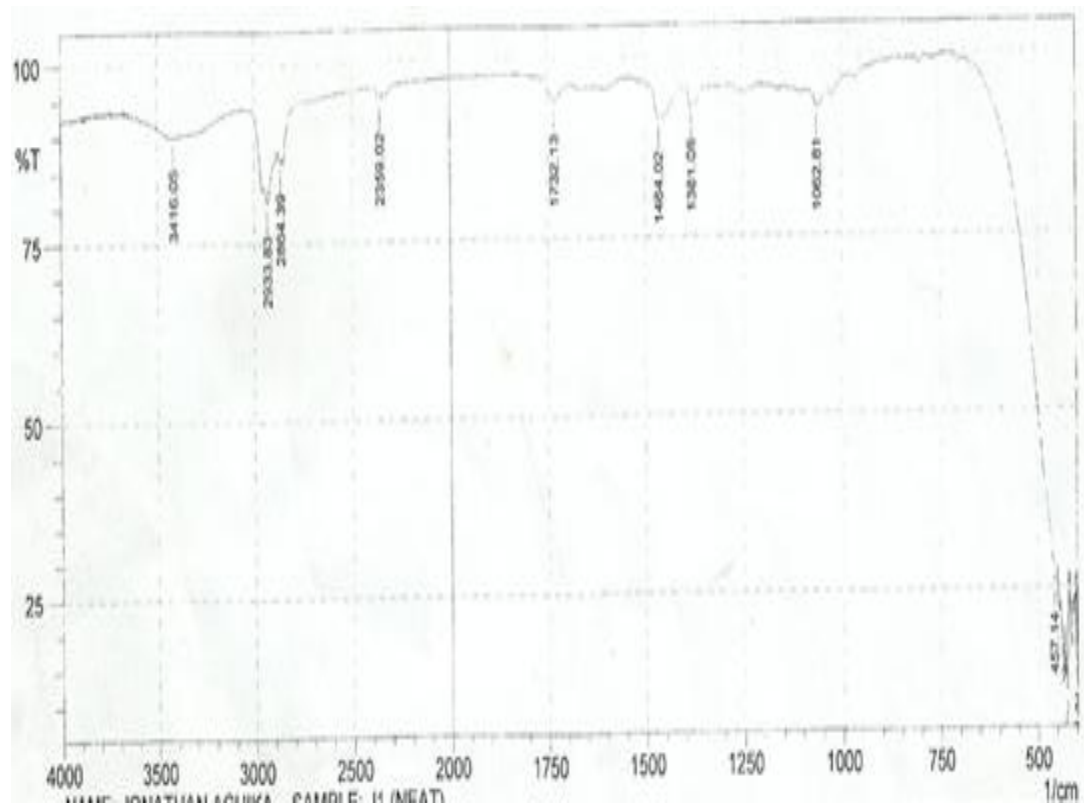


Figure 1.0: IR of AEPE

4.5 Chemical test on AEPE

From the Salkowski reaction carried out on AEPE a reddish color in the upper chloroform layer appeared indicating presence of steroidal nucleus (Harborne, 1973). Also from the Liebermann-Burchard reaction carried out, AEPE turned to violet blue and finally formed green color which indicates the presence of steroids (Harborne, 1973).

4.6 Melting Point Determination

The melting point of AEPE was melting point of 144 - 146°C.

4.7 Result of the ¹H NMR spectra of AEPE

From the proton NMR spectrum (figure 2.0), 48 proton signals were observed. The signals at 0 – 2.0 ppm are due to overlapping methine (-CH), methylene (-CH₂) and methyl (-CH₃) protons. The signal at 3.51 ppm is characteristics of oxymethine proton while the signals between 5 and 6 ppm are due to olefinic protons. These three regions of signals are characteristics of steroidal nucleus (Jain and Bari, 2000).

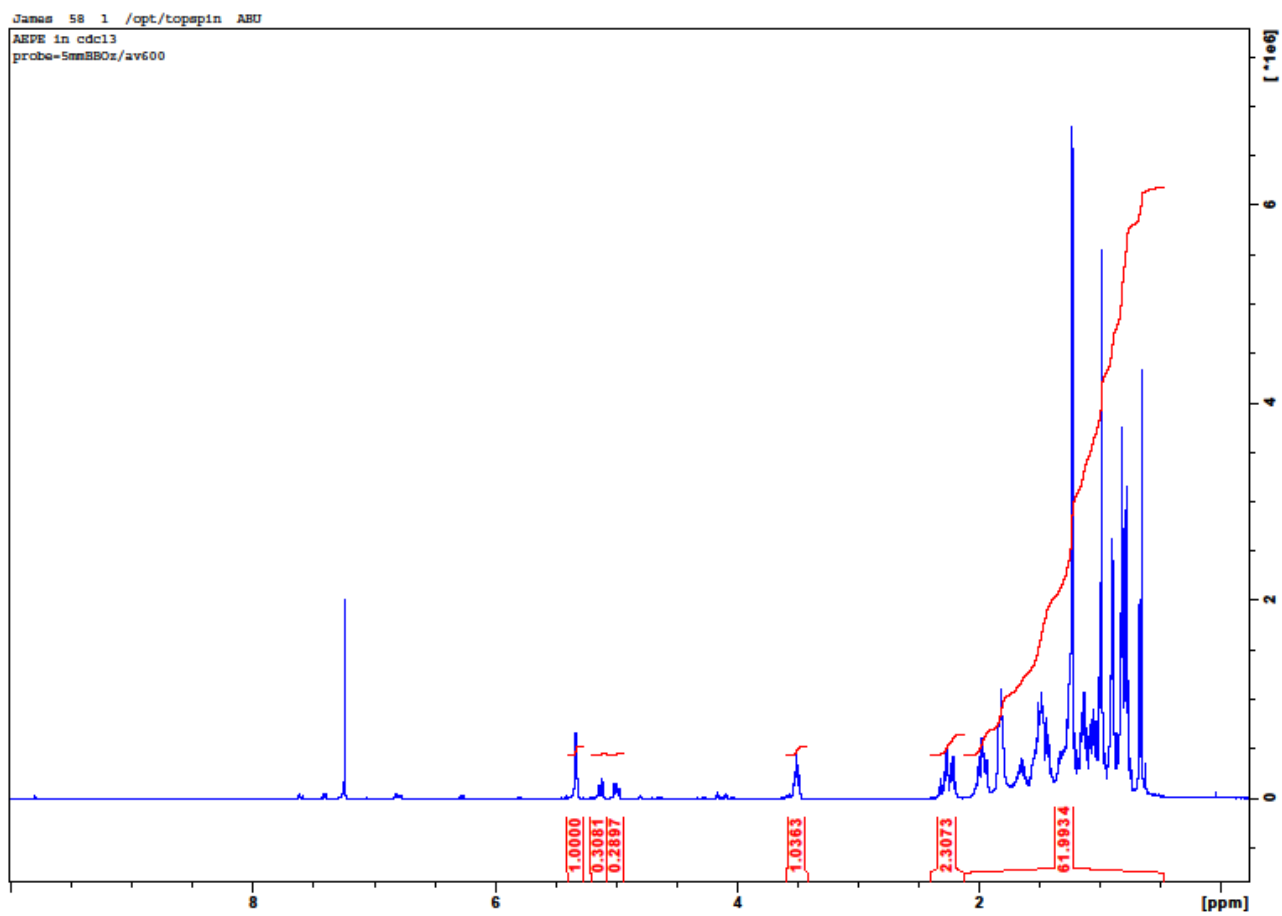


Figure 2.0: ^1H NMR of AEPE

4.8 Result of the ^{13}C NMR spectra of AEPE

The carbon NMR spectrum (figure 3.0) showed 29 carbon signals. The signals from 0 – 55ppm corresponds to methine (-CH), methylene (-CH₂) and methyl (-CH₃) carbons, and at 71.8 ppm the signal is characteristics of an oxymethine carbon. The signals at 121.7, 129.3 138.3 and 140.8 ppm are typical of 4 olifinic carbons. These three regions of signals are also typical of steroidal nucleus (Chaturvedula and Indra, 2012). Each carbon signal in ppm is recorded and compared with available literature data in table 4.9 and 4.10 respectively.

Table 4.9 ^{13}C NMR assignment for AEPE

Carbon	$\delta(\text{ppm})$	CH _n
1	38.8	CH ₂
2	29.7	CH ₂
3	71.8	CH
4	45.8	CH ₂
5	140.0	C
6	121.3	CH
7	31.7	CH ₂
8	31.9	CH
9	51.2	CH
10	37.3	C
11	23.1	CH ₂
12	42.2	CH ₂
13	42.3	C
14	56.9	CH
15	25.1	CH ₂
16	29.2	CH ₂
17	56.8	CH
18	11.9	CH ₃
19	19.8	CH ₃
20	37.7	CH
21	18.8	CH ₃
22	33.9	CH ₂
23	28.7	CH ₂
24	45.8	C
25	30.9	CH
26	21.1	CH ₃
27	19.4	CH ₃
28	21.7	CH
29	12.3	CH ₃

Table 4.10 Comparing the ^{13}C NMR and ^1H NMR spectral data of AEPE with that obtained from the literature (Pateh *et al.*, 2009)

Position	δ_c (Reference)	δ_c (AEPE)	δ_H (Reference)	δ_H (AEPE)
1	37.3	38.8	1.90	1.90
2	31.6	29.7	1.56	1.30
3	71.8	71.8	3.58	3.50
4	42.3	45.8	2.30	2.30
5	140.8	140.0		
6	121.7	121.3	5.40	5.39
7	31.9	31.7	1.50	1.51
8	31.9	31.9	2.30	1.98
9	51.2	51.2	0.98	0.98
10	36.5	37.3		
11	21.1	23.1	1.50	1.28
12	39.8	42.2	2.06	2.06
13	42.3	42.3		
14	56.8	56.9	1.04	1.04
15	24.3	25.1	1.11	1.28
16	28.2	29.2	1.30	1.30
17	56.0	56.8	1.16	1.05
18	11.9	11.9	0.74	0.74
19	19.4	19.8	1.06	1.06
20	36.2	37.7	1.40	2.00
21	18.8	18.8	0.93	0.93
22	33.9	33.9	1.07	1.07
23	26.1	28.7	1.23	1.22
24	45.9	45.8	0.97	0.97
25	29.2	30.9	1.71	1.74
26	19.8	21.1	0.88	0.88
27	19.3	19.4	0.87	0.87
28	23.1	21.7	1.31	1.31
29	12.2	12.3	0.89	0.89

4.9 Result of the COSY spectra of AEPE

The COSY spectrum (figure 4.0) displayed correlation between proton signals at 2.8 ppm and 3.45 ppm, 1.45 ppm and 3.45 ppm, 4.95 ppm and 5.15ppm, while the proton signal at 1.6 ppm correlates with that at 2.25 ppm.

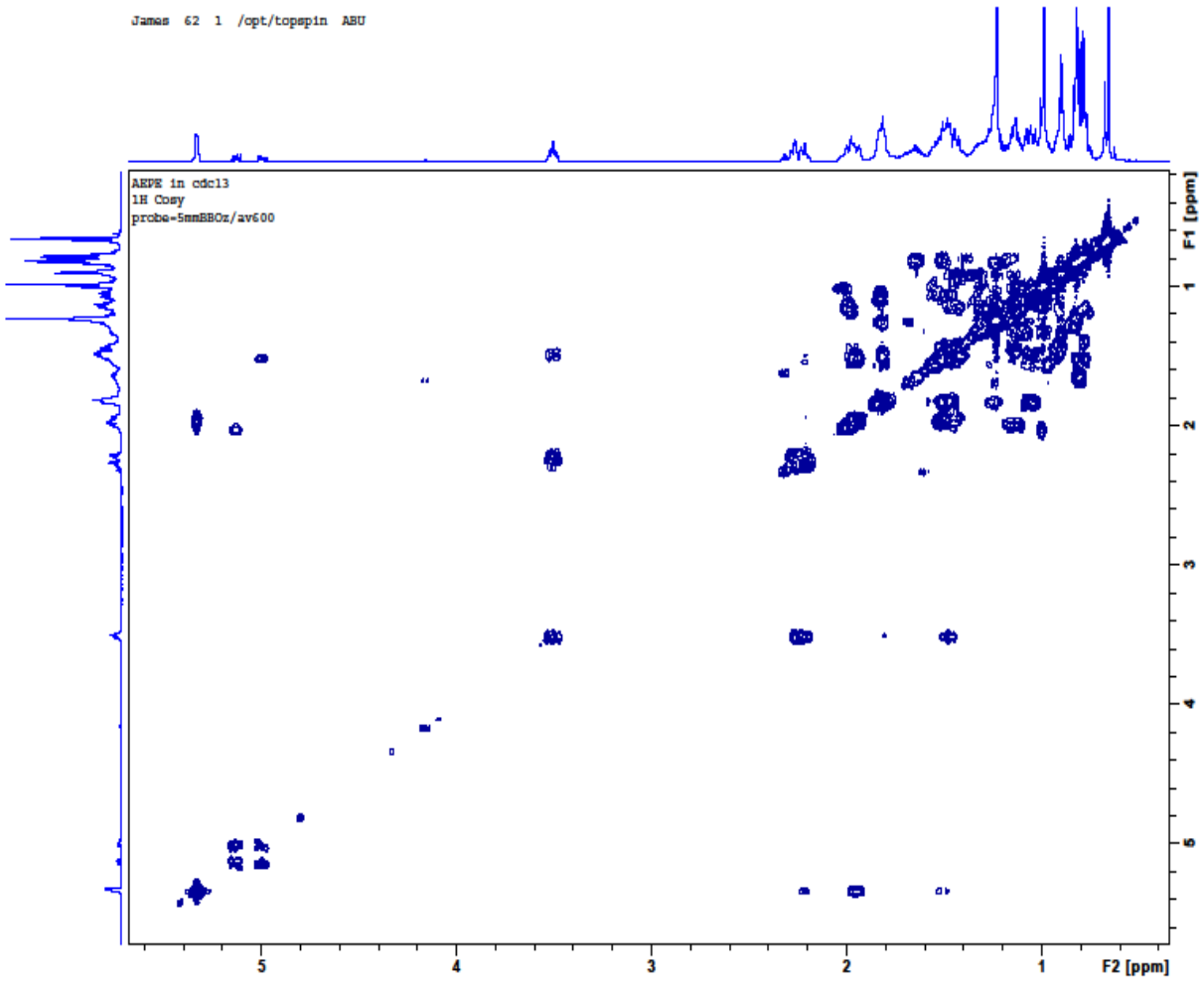


Figure 4.0: COSY ¹H OF AEPE

4.10 Result of the DEPT spectral of AEPE

The DEPT spectrum (figure 5.0) revealed the presence of six methyl carbons, ten methylene carbons; nine methine carbons and four quaternary carbons. These numbers of methyl, methylene and methine carbons are typical of stigmasterol (Chaturvedula and Indra, 2012).

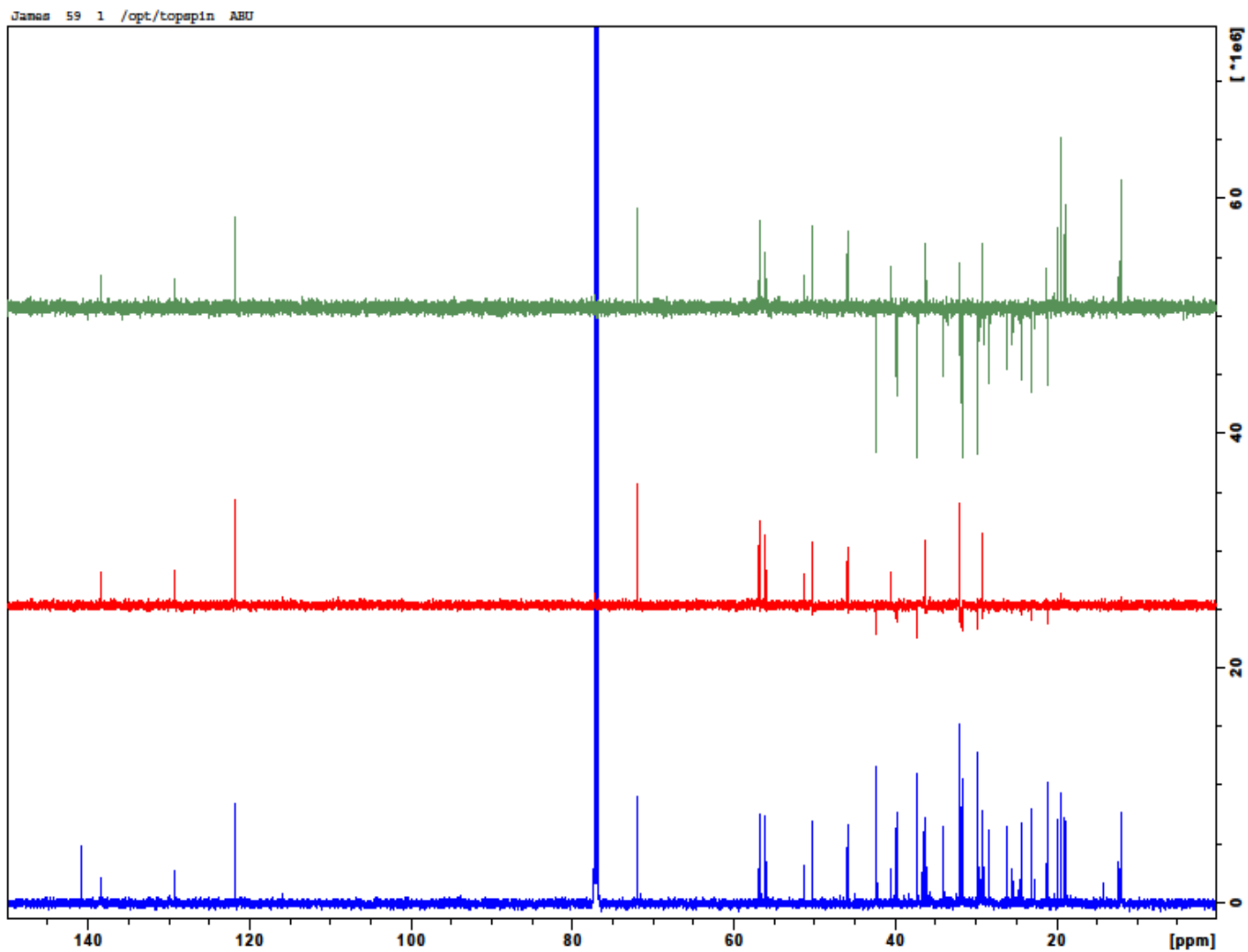


Figure 5.0: DEPT NMR OF AEPE

4.11 Result of the HMBC spectral of AEPE

The HMBC (figure 6.0) showed observable correlation which include the cross peaks between carbon signal at 71.85ppm and various proton peaks at 2.21, 1.81, 1.45 and 1.11 ppm. Observable correlation also appeared between the carbon signal at 140.1 and the proton signal at 2.21, 1.81, 1.45 and 1.11 ppm. The HMBC also showed correlation between carbon signals 120.1 ppm and the proton signal at 2.21, 1.85 and 1.45 ppm.

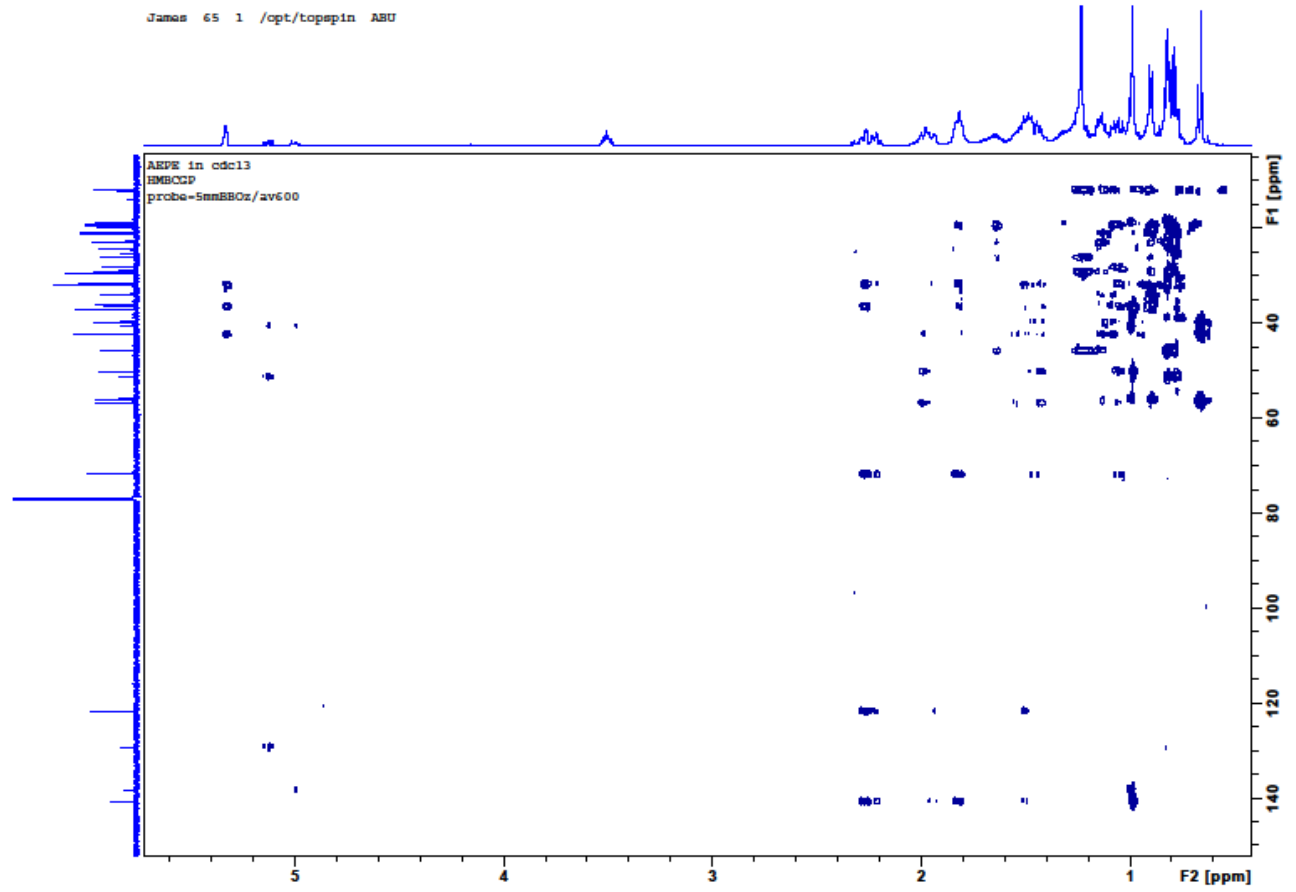


Figure 6.0: HMBC SPECTRUM OF AEPE

4.12 Result of the HSQC spectral of AEPE

The HSQC spectrum (figure 7.0) correlates the carbons and the protons signals via one bond coupling. Some important correlation observed are that of the carbon signal at 70.1 ppm which correlates with the proton signal at 3.51 ppm, the carbon signal at 120.1 ppm correlating with the proton signal at 5.41 ppm and the correlation between the carbon signal at 57.00 ppm and the proton signal at 1.04 ppm.

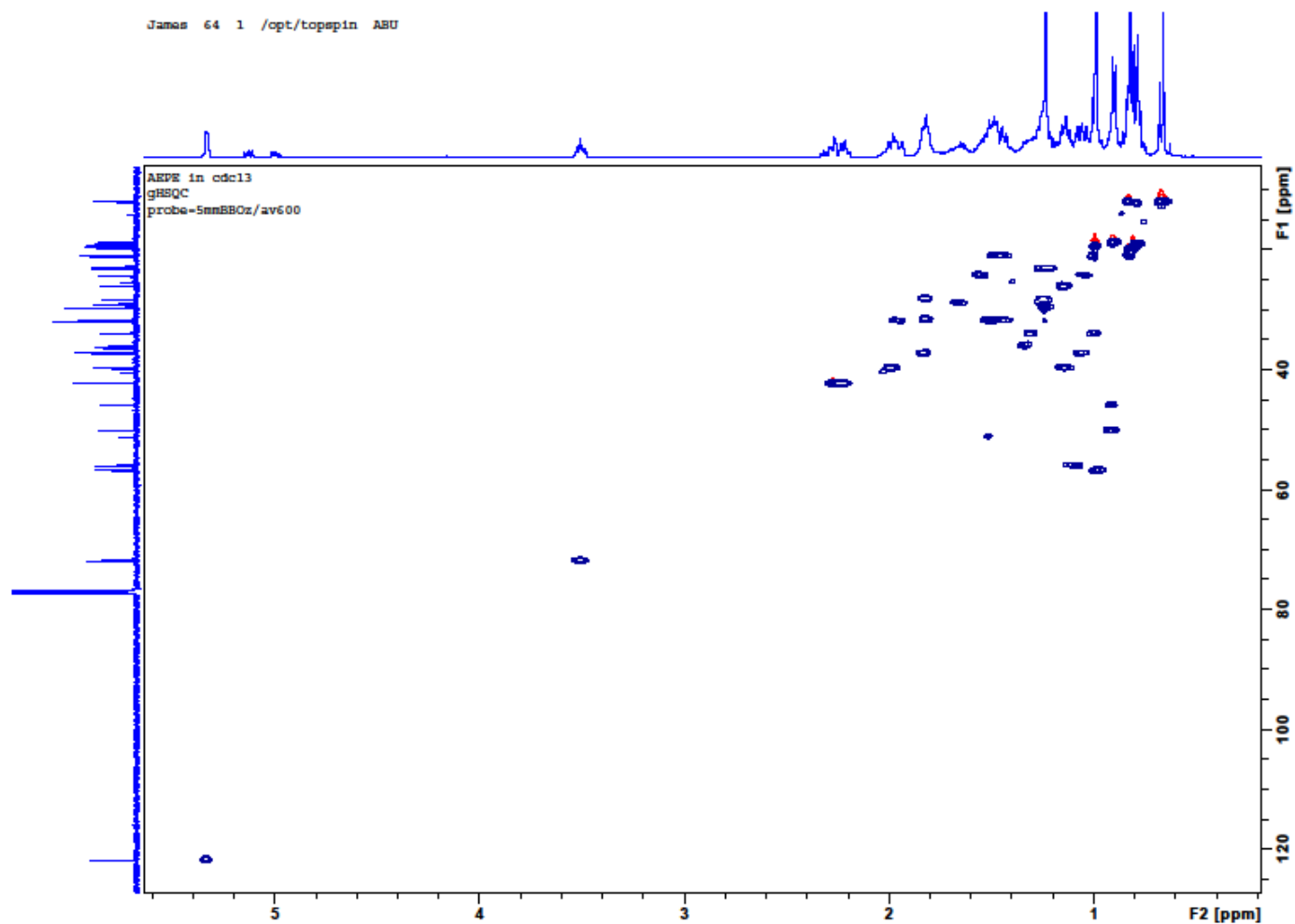


Figure 7.0 HSQC SPECTRUM OF AEPE

James 63 1 /opt/topspin ABU

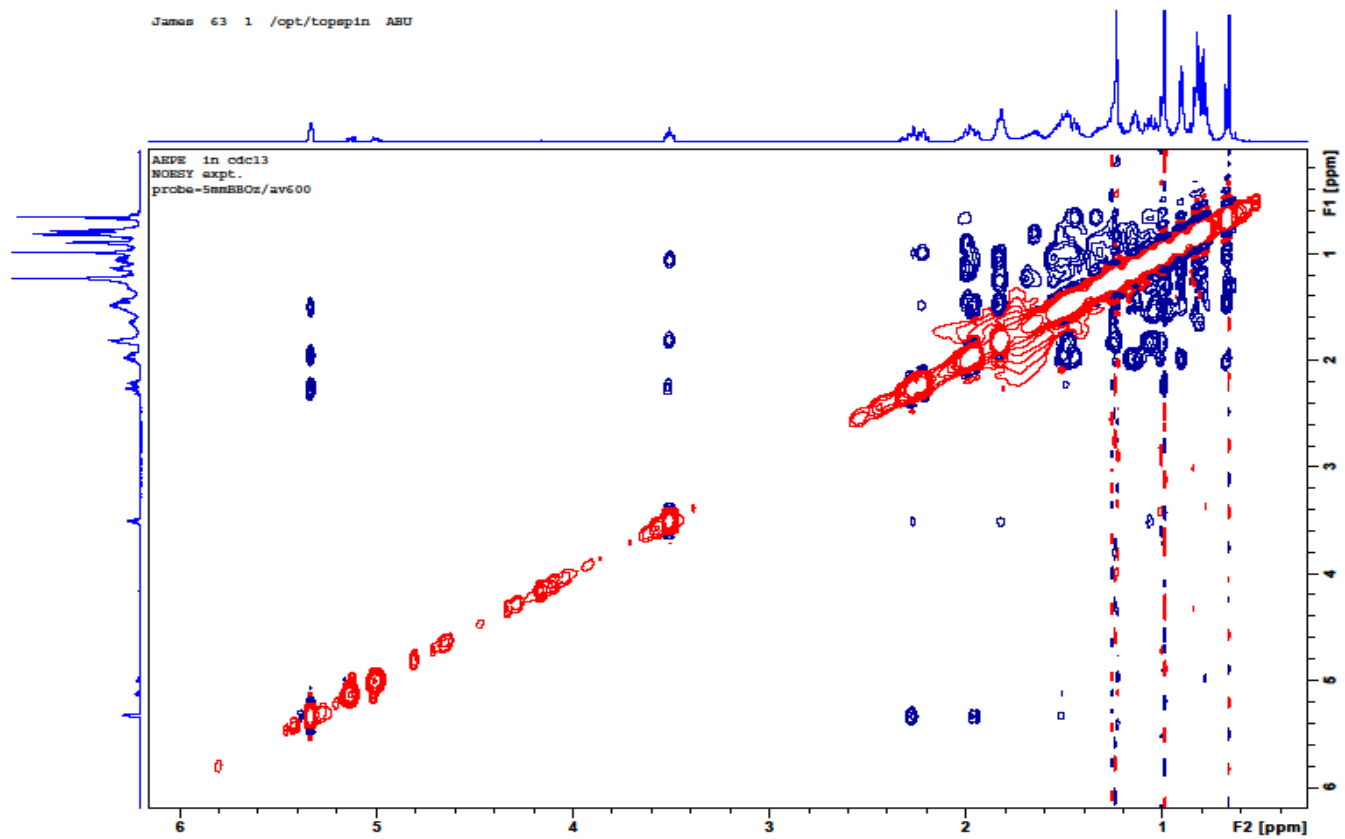


Figure 8.0: NOESY SPECTRUM OF AEPE

CHAPTER FIVE

5.0 DISCUSSION

Phytochemical screening of the crude extracts of *aeschynomene uniflora* revealed the presence of carbohydrates, steroids and triterpenes in the petroleum ether, chloroform, ethyl acetate and methanol extracts while tannins and flavonoids were present only in the ethyl acetate and methanol extracts. The presence of these secondary metabolites in plant produces some biological activity in man and animal, and it is responsible for their use medicinal as herbs (Sofowora 1993).

Standard purification technique such as column chromatography and preparative thin layer chromatography led to the isolation of a compound AEPE which gave a positive test for the Salkowski reaction, indicating the presence of a steroidal nucleus in the compound.

The broad absorption peak at 3416cm^{-1} is typical of a hydroxyl group, also the intense band at 2933cm^{-1} can be assign to aliphatic stretch. This is an indication that AEPE might be a steroid (Arjun *et al.*, 2010).

It was observed that in the $^1\text{H-NMR}$ spectrum of AEPE, the oxymethine (H-3) proton appeared as a triplet of a double doublet (tdd) at δ 3.51 ($J = 4.5$ and 1.0 MHz) and H-6 olefinic proton showed a multiplet at δ 5.14. Two olefinic protons appeared downfield at δ 4.16 and δ 4.14 which were identical with the chemical shift of H-22 and H-23, respectively of stigmasterol (Habib *et al.*, 2007; Jain and Bari, 2010). Six methyl protons also appeared at δ 1.21, δ 1.17, δ 1.03, δ 0.99, δ 0.97 and δ 0.90 (3H each, singlet, CH_3). These assignments were in good agreements with reported values (Jain and Bari, 2010; Pateh *et al.*, 2008).

The ^{13}C NMR in combination with the DEPT and HSQC spectra of compound AEPE has shown recognizable (olefinic carbon) signals at 140.8 and 121.7 ppm, which are assigned C5 and C6 double bonds respectively. The δ value at 71.0 ppm is due to (C3 β hydroxy group) oxymethine carbon (Chaturvedula and Indra, 2012). The signals at δ 19.4 and 11.9 ppm corresponds to angular carbon atom (C19 and C18 respectively).

DEPT experiment revealed the presence of six methyl carbons at C-18, C-19, C-21, C-26, C-27, and C-29; ten methylene carbons at C-1, C-2, C-4, C-7, C-11, C-12, C-15, C-16, C-22 and C-23; nine methine carbons at C-3, C-6, C-8, C-9, C-14, C-17, C-20 and C-25 and four quaternary carbons at C-5, C-10, C-13 and C-24. The de-shielded signal at δ c 71.8 was due to C-3 with a hydroxyl group attached to it (Table 4.7) (Habib *et al.*, 2007). Based on the analysis above, the structure of AEPE was determined as stigmseterol. The physical and spectral data of AEPE were in good agreement with those reported in literature for stigmasterol. (Jain and Bari, 2010; Pateh *et al.*, 2008).

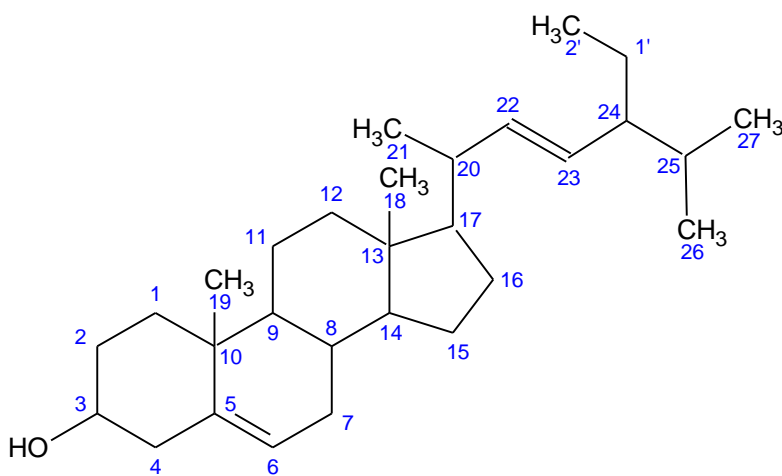


Figure: 9 AEPE: 3 β , 22E-Sigmasta-5, 22-dien-3-ol (C₂₉H₄₈O, 412.7 g/mol)

The compound isolated from the pet ether extract of the aerial part of *Aeschynomene uniflora* was a steroid. A steroid is a type of organic compound that contains a characteristic arrangement of four cycloalkane rings joined to one another. Examples of steroids include the dietary lipid cholesterol, the sex hormones, estradiol and testosterone (Desmond and Gribaldo, 2009). The steroids are among the most widely used class of drugs and their role in the therapy of pulmonary, inflammatory, dermatological and oncological diseases has been well described (Grover *et al.*, 2007). Isolated steroids have been reported to possess pharmacological activity such as antifungal, antibacterial and antioxidant activity (Govindappa *et al.*, 2011).

The ability of the crude extracts to inhibit the growth of several bacterial and fungal species is an indication of the broad spectrum anti-microbial potential of *Aeschynomene uniflora* which makes the plant a candidate for antibiotic and antifungal drugs. In the present study, the ethyl acetate extract of aerial part of *Aeschynomene uniflora* showed maximum inhibitory activity compared to others. This could be due to the fact that the ethyl acetate crude extracts of the aerial part of *Aeschynomene uniflora* might contain more number of secondary metabolites responsible for the antimicrobial activity and inhibition of the growth of microbes. It can be inferred that due to the antimicrobial effects of the ethyl acetate extracts against three Gram-positive human pathogens — *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus pyogenes*, it may be used for the topical treatment of skin disorders like acne, pimples and seborrheic eczema. Systemic fungal infections (fungemias) including those by *C. albicans* have emerged as important causes of morbidity and mortality in immunocompromised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation). Thus the sensitivity of the gram negative bacteria *candida albicans* to the petroleum ether, chloroform, ethyl acetate and methanol implies that the plant could be used to manage immunosuppressive ailments.

The sensitivity of *K. pneumonia* to the isolated compound is favorably comparable to the standard drug ciprofloxacin which is in good agreement with the reported antibacterial activity of plant metabolites which possesses steroidal nuclei (Govindappa *et al.*, 2011). The isolated compound could therefore be a potential source of drugs for managing pneumonia. The sensitivity of the isolated compound (stigmasterol) against *Staphylococcus aureus* indicates that the chemical compounds can be further developed for the fight against this microorganism and the use of the plant in the treatment of boils and skin rashes is justified since the fungi is responsible for such illness. The sensitivity of *Escherichia coli* to the isolated compound implies that the compound is a potential source of antifever, antidiarrhea, antinausea and antifatigue drugs.

CHAPTER SIX

6.0 Conclusion.

The results from this investigation indicates that the plant extracts offer significant potential for the development of novel antimicrobial therapies and treatments of several diseases caused by microorganisms. Based on the findings in this work it can be concluded that stigmasterol was isolated from *Aeschynomene uniflora*, with antimicrobial activity on *S. aureus*, *B. subtilis*, *Streptococcus pyogenes*, *Klebsiella pneumonia* and *Candida krusei*.

Stigmasterol plays a role in reducing [inflammation](#), which may be because it is a precursor to chemical compounds which can limit inflammatory processes. Sterols like stigmasterol have also been recommended for their cholesterol lowering and cancer prevention abilities. Also stigmasterol has being used by pharmaceutical industry to make synthetic progesterone for medical use.

6.1 Recommendation

More compounds should be isolated from other sub fractions and subjected to biological and pharmacological activity to investigate the medicinal potential of *A.uniflora*.

Detailed antimicrobial studies exploiting other resistance strain of microorganism should be carried out on AEPE and other crude extracts to ascertain the potency and mechanism of its antimicrobial activity.

More scientific evaluation and clinical trials should be done to establish the therapeutic efficacy of stigmasterol.

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